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THE MECHANISM OF THE ELIMINATION OF PHENOLSULPHONEPHTHALEIN BY THE KIDNEY—A PROOF OF SECRETION BY THE CONVOLUTED TUBULES *

By E. K. Marshall, Jr., and J. L. Vickers

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Introduction

Before presenting the results which have been obtained in the present investigation, it is necessary to review briefly the present status of the problem of renal secretion. So many summaries have been written in recent years concerning this problem that it would seem hardly necessary to review the literature in detail. An almost overwhelming amount of evidence points to filtration through the glomerular capsule, and its necessary consequence of reabsorption of water and certain solids by the tubules as processes which take place in the formation of urine by the kidney. The literature and evidence for filtration and reabsorption is thoroughly reviewed by Cushny [1] in his monograph on the secretion of urine. Richards,[2] in his recent Harvey lecture, has also reviewed the evidence and presented new data of his own in support of filtration. Marshall and Crane [3] in an article

* This paper was read before the Johns Hopkins Medical Society, October 16, 1922.

appearing a few months ago have discussed the present status of the problem.

Numerous investigators [4] have advocated, in addition to filtration and reabsorption, secretion by the tubules, in their theories of urinary secretion. The evidence that the process which has been termed *secretion* takes place in the kidney is by no means convincing or satisfactory. After carefully reviewing the evidence, Cushny [1] was led to outline what he calls the "modern theory" which attempts to explain the excretion of urine entirely on the basis of filtration and reabsorption. A well-known writer [5] states in the last edition of his textbook "In fact we must acknowledge that the evidence for secretion by the cells of the convoluted tubules is very defective." The evidence for secretion in the kidney may be considered under the following heads: (1) numerous investigations by histo-chemical methods indicating an accumulation of various urinary constituents (and foreign substances eliminated mainly by the kidney) in the cells of the tubules, (2) the classical Nussbaum experiment on the amphibian kidney, (3) the finding of various kinds of granules and vacuoles in the cells of the convoluted tubules, (4) attempts to determine the site of elimination of various substances by injuring certain parts of the kidneys with poisons and then noting the effect on the elimination of these substances. The histochemical evidence dates in importance from the experiments of Heidenhain [6] with indigo carmine, which was the main support of the so-called Bowman-Heidenhain theory of renal secretion. A full discussion of the various objections which have been advanced against accepting any of these lines of evidence for secretion as proof has been recently given by Cushny [1] in his monograph, and need not be repeated here. Evidence which is more or less indirect in nature has also accumulated and is probably more easily explained on a theory of filtration, reabsorption, and secretion than on one which neglects the last named process. However, it is possible to reconcile these observations with a theory of filtration and reabsorption alone.

Accepting, then, filtration and reabsorption as processes which take place in the excretion of urine by the kidney, the problem which must be settled definitely before much further progress can be made is whether these alone are in operation or if secretion and possibly other processes take place. During the past five or six years, we have been interested in this problem and have collected data, which although more easily explained by filtration, reabsorption and secretion, could still possibly be also explained by the first two processes alone.[7] However, the evidence which is presented in this communication would seem to prove definitely that the elimination of phenolsulphonephthalein by the kidney cannot be explained entirely on a filtration and reabsorption theory but that the addition of a process of secretion of the substance by the convoluted tubules is necessary.

Methods

All of our experiments were performed on dogs anæsthetized with paraldehyde. A tracheal cannula was inserted for artificial respiration, and a cannula in the carotid artery for taking a blood pressure tracing. The ureters were exposed through the flank without opening the peritoneal cavity, and cannulated. Injections were made through a cannula inserted into a branch of the saphenous vein. The spinal cord was exposed at the atlanto-occipital junction. In order to be sure that the elimination of urine had ceased it was necessary to reduce the blood pressure below 40 mm. of mercury. Simple section of the cord accomplished this purpose in only one or two instances where the initial arterial pressure of the animal was low. In general, it was found necessary to destroy a large portion of the thoracic cord to reduce the pressure to this level.

Phenolsulphonephthalein was determined in the urine by comparison with a standard solution in a Duboscq colorimeter after the addition of alkali to both samples. Considerable difficulty was encountered in accurately determining the amount of phthalein in samples of blood and tissues. Extraction with boiling water and precipitation of the proteins with the precipitants in ordinary use yielded only a fraction of the total substance present. The method finally adopted and repeatedly checked as regards accuracy was as follows: Blood, plasma, or serum was allowed to flow slowly with shaking into 9 volumes of 95 per cent alcohol made slightly acid with acetic acid. An aliquot portion of the filtrate was made alkaline with a few drops of concentrated alcoholic potash and compared with a standard solution of phthalein in alcohol, which had also been made alkaline with alcoholic potash. If proper precautions are taken to prevent evaporation, just as accurate comparisons can be made as in aqueous solutions. A weighed portion of the fresh tissue was macerated and transferred to about 10 volumes of 95 per cent alcohol in a flask. This was usually allowed to stand overnight. The solution was filtered with the aid of a suction pump, and the residue was repeatedly macerated and extracted with alcohol until the extractions gave no trace of pink color with alkali. In the case of tissues other than the kidney, 3 or 4 extractions were sufficient, but in extracting kidney tissue 15 to 20 extractions were needed and the total volume of alcohol used was frequently 2 to 3 litres for about 15 grams of tissue. The extracts were measured, made alkaline with a solution of alcoholic potash, and compared colorimetrically with an alcoholic standard of phthalein. With the exception of samples of liver, the colors could be compared satisfactorily. The alcoholic extracts of liver were treated with colloidal iron and sodium sulfate and filtered. It was found that none of the phthalein was lost under these conditions.

Ultrafiltration was carried out through a collodion membrane under a pressure of 100-120 mm. of mercury. The brands of collodion known as "Parlodion," "Necolodine," and "Biolodion" were all used. The collodion is made by dissolving 6 grams of the material as furnished by the manufacturer in 75 c.c. of a mixture of absolute alcohol and ether. Test-tubes are coated with this mixture, allowed to drain and dry for about 30 minutes, then recoated with the mixture, and allowed to drain and dry for 30 to 60 minutes. They are filled with water, the edges loosened and the sack easily removed. Ultrafiltration of plasma can be carried out through these sacks without any protein appearing in the filtrate. It was found necessary to discard the first few cubic centimeters of filtrate as these were more dilute than the later portions.

RESULTS

If phenolsulphonephthalein is injected into a dog, the animal killed shortly after the injection, and the distribution of the substance studied, the kidneys are found to contain a much higher concentration than the blood or other tissues. However, since urine containing phthalein in much greater concentration than the blood has been eliminated, and since the kidneys contain an unknown quantity of this urine, this does not indicate that the kidney tissue contains the substance in greater concentration than the blood and other tissues. To study this question we have injected phenolsulphonephthalein into dogs where the elimination of urine had been abolished by the low blood pressure incident on section and partial destruction of the spinal cord. In all these experiments the high concentration of phthalein in the kidneys compared to the blood and other tissues was very noticeable. As shown in the tables given below, the kidneys may contain as much as 35% of the total amount of phthalein injected although they make up only about 0.6-0.7% of the body weight. The concentration of phthalein in the kidneys is many times greater than in the blood or other tissues. The experiments given below are examples of several which we have carried out.

Experiment 1. Dog V63, male, 16.1 kilos.

At 10:30, ureters cannulated, urine flowed from both. At 11:10-11:40, spinal cord sectioned, and partially destroyed. At 11:41, 180 mgm. of phenolsulphonephthalein in 30 c.c. of water injected intravenously. Absolutely no flow of urine from either ureteral cannula. At 11:59, animal bled to death from the carotid, and samples of blood and tissues taken for analysis. The following table gives the amount and concentration of phthalein in the various tissues. With the exception of the kidneys, the percentage weight of the various tissues in relation to body weight are average figures taken from Wahlgren.[8]

Tissue	% Body Wt.	Phenolsulphonephthalein mgm. %	Total present mgm.
Blood	7.6	3.0	34
Serum	—	5.2	—
R. Kidney	0.55	48	40
L. Kidney		43	
Liver	3.6	2.3	13
Intestine	8.2	0.8	10
Muscle	42.8	0.8	54

Calculation indicates recovery of 151 mgm. of phthalein of the 180 mgm. injected, which is very satisfactory in view of the error in the relative weights of tissues involved in such a calculation.

Experiment 2. Dog V64, male, 15.7 kilos.

At 10:30 ureters cannulated. At 11:00 cord cut and partially destroyed. It was necessary to reduce the blood pressure to 35 mm. of mercury before the flow of urine ceased entirely. At 12:14, 180 mgm. of phthalein injected intravenously. No urine flowed after the injection. At 12:29, animal bled to death and samples of blood and tissues taken for analysis.

Tissue	% Body Wt.	Phenolsulphonephthalein mgm. %	Total present mgm.
Blood	7.6	3.6	40
Serum	—	4.8	—
R. Kidney	0.66	62	62
L. Kidney		57	
Liver	3.6	5.7	29
Intestine	8.2	1.5	19
Skeletal Muscle	42.8	0.5	33
Spleen	—	trace	—
Brain	—	faint trace	—

Calculation indicates a recovery of 183 mgm. of the 180 mgm. injected which is sufficiently close correspondence considering the variation in the relative weights of the different tissues in various dogs.

Experiment 3. Dog V14, 9.9 kilos.

At 10:14, 150 mgm. of phenolsulphonephthalein injected intravenously. At 10:40-10:50, cord cut and partially destroyed, blood pressure 30-35 mm. of mercury. At 10:56, left kidney extirpated; one hour later, right kidney removed. Not a trace of urine flowed after removal of the left kidney. The left kidney contained 43, and the right 42 mgm. per cent of phthalein.

Experiment 4. Dog V66, male, 6.6 kilos.

At 10:40-12:00, spinal cord cord cut and partially destroyed. At 12:15, 120 mgm. of phenolsulphonephthalein in 20 c.c. of water injected intravenously. At 12:30, left kidney extirpated; forty-five minutes later, right kidney removed. Left kidney contained 44, and right 50 mgm. per cent of phthalein.

As absolutely no urine flowed from the ureters in the above experiments, it is probable that phenolsulphonephthalein is either concentrated in the renal cells or transferred through the renal epithelium to the lumen of the tubules while no urine is being eliminated. As it is conceivable that filtration may be taking place in the glomeruli under the above conditions and all the fluid be reabsorbed in the tubules, these experiments are suggestive but not conclusive of elimination by a process other than filtration. On this hypothesis, however, filtration must cease in ten or fifteen minutes (when the phthalein content of the kidney ceases to increase) or phthalein be filtered and then reabsorbed with the water at exactly the same rate as that at which it is being filtered. Both of these assumptions would seem to be highly improbable. Evidence of another kind indicates that the phenolsulphonephthalein in the kidney is not in the lumen of the tubules or at least mainly in the cells. If one observes a kidney after longitudinal section from one of the above experiments, the contained phthalein is evident by a brilliant yellow color. It is easily noted, however, that the yellow color is confined to the cortex and that it is absent in the medullary rays of the cortex. Examination of a fresh section of such a kidney with the aid of an arc lamp and binocular microscope reveals the yellow color only in the convoluted tubules. Further examination of a teased preparation shows that the phthalein is apparently confined to the cells of the convoluted tubules and is absent from the lumen. It is, therefore, obvious

that filtration cannot account for the high concentration of phthalein in the kidney, but that it is specifically taken up from the blood by the cells of the convoluted tubules.*

Several facts which have been observed during the course of the present investigation have suggested that all of the phenolsulphonephthalein may not be in a free condition in the plasma; that is, available for filtration or diffusion. The treatment of an aqueous solution of phenolsulphonephthalein with colloidal iron does not remove any of this substance. If, however, the phthalein is added to defibrinated blood which is then coagulated with heat and acetic acid and treated with colloidal iron, only about 25 per cent of the phthalein is recovered in the filtrate. Trichloracetic acid, which is frequently used as a protein precipitant for blood, yields when added to blood containing phthalein only a portion of the phthalein in the filtrate. The aqueous humor has been shown to contain substances which are known to be free in the plasma, in about the same concentration as the plasma,[9] but contains much less phthalein than the blood plasma. When one recalls the older work of Bechold showing that certain phenols are largely bound in the plasma,[10] and the newer investigations of de Haan and Van Creveld [11] indicating that fluorescein is partially bound in plasma, it is reasonable to expect that a chemically related substance like phenolsulphonephthalein would be bound by the blood colloids.

The fact that all the phenolsulphonephthalein in the blood is not free and available for filtration has been determined by ultrafiltration. Plasma or serum containing phthalein, or taken from dogs into which the substance had been injected, has been filtered under a pressure of 100-125 mm. of mercury through collodion membranes. Only a portion of the total phthalein is present in the filtrate, while an aqueous solution of phthalein containing no colloids and treated in the same way yields a filtrate containing the same concentration of the substance as the original solution. The amount of phthalein which is free in the plasma appears to depend on the hydrogen-ion concentration.

A solution of phenolsulphonephthalein, made up in water containing a buffer mixture (Ph. 7.4) to contain 3 mgm. per cent, was ultrafiltered. The first 3 c.c. of the filtrate was 82 per cent as concentrated as the original; the second 3 c.c. was 100 per cent as concentrated; and the third 3 c.c. read 102 per cent against the original solution as a standard.

Serum taken from an animal into which phthalein had been injected was found to contain about 4 mgm. per cent of phthalein It was ultrafiltered with the following result. The first 2 c.c. of the filtrate was discarded, the second 2 c.c. was only 37 per cent, and the next 3 c.c. only 41 per cent as concentrated as the original serum.

A sample of serum containing 5.5 mgm. per cent of phenolsulphonephthalein was divided into two portions, one being made acid, and the other alkaline. Both were ultrafiltered, the first 3 or 4 c.c. of the filtrate being discarded. The filtrate from the acid serum showed only 20 per cent as great a concentration as the original, while in the alkaline portion the phthalein appeared to be all in a filterable condition.

In view of the fact that only a portion of the phenolsulphonephthalein in the plasma is available for filtration, the following question arises for consideration. Is there sufficient *free* phenolsulphonephthalein in the blood passing through the kidneys to account for the amount eliminated in the urine in a given time? If this can be answered in the negative, it is obvious that filtration and reabsorption alone cannot account for the elimination of this substance by the kidney. It is necessary to measure the concentration of *free* phthalein in the blood passing through the kidneys, the amount of this substance eliminated in the urine in a given time, and the quantity of blood which has passed through the kidneys in the same time. The first two of these measurements are easily made, the last is more difficult to do accurately. However, by utilizing the data which are available for the blood flow through the kidneys of anesthetized dogs, we can obtain a value which can be used in calculations in any experiment. Barcroft and Brodie [12] observed a blood flow of 2 c.c. per gram per minute, and Burton-Opitz and Lucas,[13] using a somewhat different method, found 1.5 c.c. per gram per minute. It would certainly seem that a flow of 5 c.c. per gram per minute would be a safe maximum on which to base any calculations in this respect. Experiments on dogs have clearly shown that the amount of phthalein eliminated in the urine in a period of five minutes is in excess of the amount of free phthalein calculated to have been brought to the kidney by the blood in this time. It is obvious, then, that filtration cannot account for all of the phenolsulphonephthalein eliminated by the kidney *even if all the plasma were filtered.* In fact it is very doubtful if sufficient total phthalein—free and bound—is brought to the kidney to account for the phthalein eliminated, assuming the kidney to be 100 per cent efficient in its removal. This apparent paradox is, of course, explained by accumulation of the substance in the renal cells soon after injection and before the actual experimental observations are commenced. Such a store of phthalein might be gradually eliminated in the urine.

Experiment 5. Dog V57, male, 10.8 kilos.

Ureters cannulated, a ten per cent solution of sodium sulphate injected intravenously (90 c.c.). At 2:52, 150 mgm. of phenolsulphonephthalein injected. Specimens of urine and blood from the carotid obtained as follows and analyzed for phthalein. From 2:53 to 3:03, urine collected (sample I). At 2:53-3:03 blood drawn (sample I). Urine II from 3:03:50 to 3:08:50. These specimens were analyzed and gave the following results.

* We desire to thank Dr. Warren H. Lewis for his kind assistance in examining our early specimens by these methods.

	RIGHT		LEFT	
	Volume c.c.	Phthalein mgm.	Volume c.c.	Phthalein mgm.
Urine I.....	22.5	18.7	22.0	18.2
Urine II.....	9.8	7.9	9.0	8.1

Blood I—2.86 mgm. % of phthalein.
Plasma I—4.22 " " " "

Blood I gave 32.5 per cent corpuscles with the hæmatocrit. Plasma I gave by ultrafiltration 35 per cent of the phthalein free. The kidneys together weighed 65 grams. Assuming a blood flow of 5 c.c. per gram per minute, in the five minutes during which urine sample II was collected, 1600 c.c. of blood flowed through the kidneys. Calculations from the concentration of phthalein in the blood and plasma and the hematocrit value indicate that all of the phthalein is in the plasma. Taking the value 2.86 mgm. per cent which was the concentration in the blood at the commencement of the period, calculation shows that 43.8 mgm. reached the kidneys. Of this, only 15 mgm. were free or available for filtration. Now, since 16 mgm. were eliminated by both kidneys during this time, it is obvious that filtration of all the plasma reaching the kidneys will not account for the amount eliminated. About 37 of the 150 mgm. injected were eliminated in the urine before the period for calculation was taken, which would leave 113 mgm. in the body. Taking the blood volume as 8 per cent of the body weight, we can calculate that only 23 mgm. were present in the blood, which leaves 90 mgm. in the other tissues. If the distribution of phthalein in the tissues in these experiments is the same as was found in the case of dogs with the spinal cord sectioned, about a third of the 90 mgm. was contained in the renal cells.

Experiment 6. Dog V68, male 14.3 kilos.

Ureters cannulated, a ten per cent solution of sodium sulphate injected intravenously (100 c.c.). At 3:05, 300 mgm. of phenolsulphonephthalein injected. Specimens of urine, and blood from the carotid obtained as follows, and analyzed for phthalein. At 3:16:20, blood drawn (sample I). Urine I from 3:16:20 to 3:21:20. At 3:21:50, blood drawn (sample II). Urine II from 3:21:20 to 3:26:20. At 3:26:50, blood drawn (sample III). These specimens were analyzed, and gave the following results.

	RIGHT		LEFT	
	Volume c.c.	Phthalein mgm.	Volume c.c.	Phthalein mgm.
Urine I.....	6.5	12.0	7.6	12.2
Urine II.....	12.7	11.0	14.0	11.5

Blood I—4.08 mgm. % phthalein.
Blood II—3.00 " " "
Blood III—2.22 " " "

Plasma gave by ultrafiltration 40% phthalein free. Kidneys together weighed 63 grams.

Assuming a blood flow of 5 c.c. per gram per minute, 1575 c.c. of blood passed through the kidneys in five minutes; and contained, in the period in which sample II of urine was collected, 47 mgm. of phthalein of which only 18.8 mgm. were in the free (filterable) state. Since 22.5 mgm. were eliminated by the two kidneys during this period, it is obvious that filtration of all the plasma coming to the kidneys could not account for the amount of phthalein in the urine. In the above calculation the value for the concentration of phthalein in the blood at the beginning of the period has been taken in an attempt to allow for the dead space in the kidney or the part of the urine which belongs to the previous period. Even if we assume all of the urine collected in sample II belonged to the period of sample I, which would make the kidneys contain about 27 grams of urine for a weight of 63 grams, it is still impossible to reconcile the amount of phthalein eliminated in the urine with filtration. Taking 3.5 mgm. per cent to represent the average concentration in the blood during the first period, we find that 55 mgm. of total phthalein reached the kidneys, and that only 22 mgm. of this was in a filterable condition.

In these experiments, the observations were begun at least ten minutes after the injection of phthalein had been given. If the observations are made within a few minutes after the injection of the substance, the results are not the same, apparently because sufficient time has not been allowed for the accumulation of phthalein in the renal cells. This would agree with certain observations which we have made, indicating that the maximum elimination after intravenous injection of phthalein does not occur as soon as the substance appears in the urine (when the blood concentration is probably at its height) but some time later.

DISCUSSION

The results which have been given above indicate definitely that the passage of a colloid-free filtrate of plasma through the glomerular capsule is insufficient to account for the amount of phenolsulphonephthalein found in the urine on the basis of the amount of free (filterable) phthalein in the blood. It is obvious that the assumption that all of the plasma could be filtered is incorrect; the increasing concentration and hence osmotic pressure of the proteins would soon bring filtration to an end. Sufficient allowance has, therefore, been made for the two factors in the calculation which are not accurately known—the volume flow of blood through the kidney and the amount of urine present in the tubules and pelvis of the organ. Should our figures for these values be correct or should the kidney not remove completely the phthalein entering it in the arterial blood, it is also obvious that there is not sufficient total phthalein in the blood at the time of the experiment to account for that which has been eliminated in the urine. The only conclusion which can be drawn is that the substance has been stored in the renal cells at the time of injection and is slowly given out in the urine.†

The demonstration of the ability of the kidney to pick out phenolsulphonephthalein from the blood and con-

† One might venture a partial explanation of the concentration of the phthalein in the cells of the convoluted tubules as follows. The phthalein which is free in the blood diffuses into the tubule cells, but is immediately adsorbed by the kidney colloids, more free phthalein is formed in the plasma and this diffuses into the cells and is removed by being bound. This assumes that the colloids of the cells of the convoluted tubules have a greater adsorptive power for the substance than the plasma colloids, and only explains the concentration in the renal cells.

centrate it when no urine is being formed is strong evidence for a similar condition of affairs when urine is being secreted. The microscopic localization of the phthalein in the non-secreting kidney in the cortex and indeed in the cells of the convoluted tubules leaves but little doubt that this is the part of the tubule which is concerned with the secretion of the substance. Of course, the free phthalein present in the blood will also be eliminated along with all the other non-colloid constituents of the plasma in the glomerular filtrate. Experiments based on the production of a localized injury to the kidney by means of poisons have pointed to the convoluted tubules as the main seat of phthalein elimination.‡ This is well shown in the case of tartrate nephritis, as studied by Underhill and Blatherwick [14] and by Potter and Bell.[15] The inconclusiveness of such evidence without additional support, as we have obtained, however, has already been discussed.

The problem which was presented in the introduction to this paper would then appear to be definitely settled, and satisfactory evidence would seem to exist that the processes which have been designated filtration, reabsorption, and secretion all play a rôle in the elimination of the urine. The synthesis of hippuric acid by the kidney has long been known, but it has been recently found that this is not confined to the kidney [16] so that whether the kidney eliminates only hippuric acid preformed in the blood or also that which it synthesizes is not known. However, the quite recent work of Nash and Benedict [17] indicating that the kidney produces the ammonia of the urine, and that of Behre and Benedict [18] suggesting that creatinine may be formed by the kidney, would necessitate ascribing in addition to secretion the process of synthesis to some of the epithelial cells. How far one can at present apply these processes to a description of the process of urine formation, with attention to what part each process bears to the elimination of the different urinary constituents, has been outlined in a recent paper by one of us,[3] and will be further discussed in an article to be published in the near future.

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‡ In some of these experiments and occasionally in clinical cases of renal disease, it has been observed that when phenolsulphonephthalein is injected in the usual way, not a trace is excreted by the kidney. A considerable amount of urine may, however, be eliminated. If the phthalein is present in the blood in a filterable condition, it must be present in the glomerular filtrate, and hence must disappear by reabsorption in the course of the passage of this filtrate along the tubules. Another explanation that might be suggested is that no free phthalein exists in the blood in these cases but that it is all in a bound and non-filterable form.

ADDENDUM.—After this paper had been sent in for publication, an article appeared by de Haan (Jour. Physiol., LVI, 444, 1922) which has a direct bearing on our results. He studies the condition of several dyes in the plasma in relation to their elimination by the kidney, and finds that all of the colored substances examined are bound to some extent in the plasma according to the evidence furnished by ultrafiltration. Calculating the concentration of the dyes by the kidney on the basis of the amount of the free substance present in the same volume of plasma and urine, he finds these substances are concentrated to a much greater extent than urea. The concentration in some cases is much greater than could be explained by the passage of a colloid-free filtrate of plasma through the glomeruli and reabsorption of fluid by the tubules. We believe that de Haan's experiments furnish proof of secretion, but he interprets them otherwise. He assumes that the glomerular filtrate normally contains colloids and that both the free and bound dye is filtered through the glomerulus, the dye being set free from the protein in its course down the tubule, and the protein reabsorbed. This explanation is not only supported by no evidence, but is opposed to all of our knowledge of the glomerular fluid. The presence of protein in the fluid in the glomerular capsule is indicated in sections of kidneys fixed in boiling water or with the usual fixatives, when the urine contains albumin; in normal kidneys no indication of protein in the glomerular fluid is obtained by these methods (Posner, Virchows Arch. LXXIX, 311, 1880; Seelig; Arch. f. Exp. Path. u. Pharm., XXVIII, 265, 1891). Furthermore, the fluid withdrawn from the glomerular capsule of the frog's kidney is free of protein (Wearn; Amer. Jour. Physiol., LIX, 490, 1922).

A point of particular interest to us in de Haan's paper is a single experiment which he gives on phenolsulphonephthalein

(phenol red). He finds that the ultra-filtrate of plasma containing this substance is less than 2 per cent as concentrated as the original plasma, while in our experiments we found between 30 and 40 per cent the concentration in the ultra-filtrate. Since de Haan used rabbits, and our work was carried out on dogs, it seemed possible that the difference in species might explain the discrepancy. We have repeated and extended our experiments on the ultra-filtration of dog's plasma and serum containing phthalein, and also performed a few experiments with rabbit's plasma and serum. Values from 25 to 40 per cent of the concentration of the phthalein in the plasma or serum have been obtained in the ultra-filtrate in the case of dogs, while rabbit's plasma or serum containing phthalein yields an ultra-filtrate containing only 2.5 to 5 per cent of the concentration of the original phthalein. It thus appears that 60 to 75 per cent of the phthalein in dog's plasma is bound or non-diffusible, and 95 to 98 per cent is in this condition in rabbit's plasma. A single experiment indicated that in human plasma 34 per cent of the phthalein is bound. These findings indicate that it is very unsafe to draw conclusions as to the condition of substances in the blood of one species from data obtained with the blood of another. Many of the discrepancies found in the literature in regard to the amount of the various crystalloids present in the bound form in the plasma may possibly be due to the use of the blood from different species of animals. These discrepancies are quite noticeable in respect to the amount of calcium found to be in a bound form by different observers (for literature and discussion, see Neuhausen and Marshall, Jour. Biol. Chem., LIII, 365, 1922).

THE LIPOIDS OF MATERNAL AND FETAL BLOOD AT THE CONCLUSION OF LABOR

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Principally to secure information regarding the activity of the placenta which links the nutritional processes of mother and fetus, specimens of blood obtained from each simultaneously have been analysed and the results compared. Data of this kind indicate that the placenta is permeable to glucose, to amino acids, and also to the end-products of carbohydrate and nitrogenous metabolism. On the other hand, the estimation of the fat and the lipoids by the familiar methods of Bloor leads to the opposite conclusion. In a brief statement we have already expressed the view that the human placenta is impermeable to these substances and consequently that they are not included among the materials the mother contributes toward fetal development and growth. This impression has been confirmed by a more extensive series of observations which are the basis for this report.

FAT

During the latter part of pregnancy the fat and lipoids gradually increase, and at full term there are approximately 900 mgs. of fat per 100 c.c. of maternal blood—a quantity at least one-third greater than the accepted normal for women. The cause of this increase is not known; its purpose, probably, is to prepare the mother for successful lactation. It would not seem intended for immediate use, for the respiratory quotient during pregnancy indicates only the ordinary utilization of fat: and clinical evidence actually points toward a somewhat lowered tolerance for this foodstuff. At a later time, obviously, the production of milk requires an appreciable supply of fat which will be derived from the body of the mother, if her diet is inadequate. It is reasonable to suppose, then, that the unusual quantity of fat in the blood during the latter part of pregnancy is explained by the transportation of this material to storage centers which may be drawn upon when the breasts become active.

It is not unnatural that some students of metabolism have assumed there was an active transportation of fat from mother to fetus through the agency of the placenta; this view harmonized satisfactorily with what we should imagine to be true, were there no evidence to the contrary. However, we now possess quite direct evidence which bears upon the problem. By methods inapplicable to human beings the facts were ascertained long ago with regard to animals readily submitted to experiment. Ahlfeld, in 1877, selected dogs for the study of this question. There was a preliminary period of starvation. Then, the dogs were fed with bacon and, after an interval of twelve hours, fat determinations made on the blood of the mother and also on the blood of the pups just removed from her uterus. A very striking difference in the specimens was apparent to the naked eye; the blood from the mother was distinguished by its milkiness, while that from the pups resembled normal blood and was unaffected by the fatty diet employed. More exact evidence was furnished by the result of chemical analysis which showed the maternal blood to be ten times richer in fat than the fetal blood. As there was time for equalization of the fat in the two circulations, if any mechanism existed for this purpose, Ahlfeld concluded that fat could not pass through the dog's placenta.

Furthermore, experiments with vital staining in guinea-pigs and rats have clearly taught that the placental transmission of fat never occurs in these mammals. After the injection of Soudan III into pregnant guinea-pigs, Gage and Gage observed the typical distribution of stained fat in the mother, yet no trace of the

dye was found in the fetus. Similar results in the case of rats convinced Mendel and Daniels that here also the placenta was an effectual barrier to the passage of fat.

There is a tendency among obstetricians to regard the action of the human placenta as exceptional, for fat droplets are demonstrable microscopically in parts of the organ and some investigators have interpreted them as food on its way to the fetus. However, more intimate studies, like those of Eden, seriously discredit such an interpretation. The droplets, he finds, are confined mainly to the outer zone of syncitium in contact with the mother's blood and appear in the neighborhood of the nuclei. This picture is not one of tissues actively engaged in fat transportation, if the intestinal wall is a criterion. While digestion and absorption are in progress, fat globules may be traced with the microscope continuously from the lumen of the gut into the lacteals. Histologically, then, there is no analogy between the intestinal and the placental villi. The isolated fatty droplets at the periphery of the latter have only a local significance. They do not travel toward the fetus and almost certainly pertain merely to the metabolism of the tissues which contain them.

The inactivity of the human placenta as an agent in fat transportation is indicated most convincingly, perhaps, by the results of blood analysis. The specimens from the mother and those from the fetus differ so constantly, yet so irregularly, as to indicate the complete independence of the two organisms with respect to the metabolism of fat. It is significant, as the one invariable feature of our results, that the maternal specimen, whether whole blood or plasma, is always the richer in both fat and lipoids.

AVERAGE VALUES FOR FAT IN MOTHER AND FETUS

Source	No Anæsthetic	Light Anæsthesia	Deep Anæsthesia
WHOLE BLOOD			
Mother	908 mgs.	862 mgs.	880 mgs.
Fetus	707	780	727
PLASMA			
Mother	942	967	960
Fetus	737	758	763

Variations from case to case, as has been said, are regularly encountered, and average values, consequently, have been computed as a matter of convenience to facilitate description. Thus, the plasma fat of the fetus ordinarily falls about 200 mgs. below that of the mother; but this difference may be as small as 75 or as great as 400 mgs. Though the results for both mother and fetus fluctuate between widely separated extremes, the maternal blood shows the greater tendency to vary in its fatty content. Among the patients studied, for example, the maternal plasma fat varied from 1240 to 800 mgs., whereas the fetal plasma fat varied from 840 to 635 mgs.

In the face of wide variations when normal obstetrical patients are selected, it is impossible to attribute any change in the blood fat to complications. Primiparæ are indistinguishable from multiparæ. The duration and the severity of labor do not, as some have thought, regularly influence the blood lipoids. Anæsthesia has no characteristic effect. Nor in the presence of toxæmia have we been able to recognize any constant departure from the usual findings.

A very large quantity of fat in the maternal blood is not associated with a corresponding change in the blood of the fetus. Not only is it true that different quantities always prevail in the two circulations, but no constant degree of difference between them exists. Whatever the standpoint taken for the study of the data collected, we are unable to recognize a relationship between the volume per cent of fat in the mother's blood and the volume per cent of fat in the blood of the fetus. Such a situation, it seems to us, signifies the complete independence of the organisms with respect to fat metabolism. The human placenta, like the corresponding organ in the dog, the guinea-pig and the rat, is impenetrable to fat. In these circumstances, obviously, fetal fat must be synthesised from some material, probably glucose, which passes readily through the placenta and is supplied by the mother in quantities proportionate to the demands of the fetus.

Lecithin

Although the composition of lecithin has been determined, its wide distribution in nature ascertained, and its fundamental part in several important biological reactions definitely established, the physiological rôle of this lipoid still remains enigmatical. Evidence is accumulating which assigns to lecithin a part in the early stage of fat metabolism. Bloor attributes its origin to a synthesis of fat and phosphoric acid effected through the agency of the bood corpuscles. In accord with that hypothesis it would not be reasonable to expect any relationship between the maternal and the fetal lecithin. This seems to be the case. Conspicuous differences in the concentration of lecithin on the two sides of the placenta indicate its impermeability to this substance and consequently the complete independence of mother and fetus with regard to lecithin metabolism.

In harmony with the quantity of fat in the mother's blood at the end of pregnancy there is also an increase in the quantity of lecithin. Bloor estimates the normal for women to be 290 mgs. of lecithin per 100 c.c. of whole blood and 190 mgs. of lecithin in the same unit of plasma. At the conclusion of labor the corresponding values for the whole blood vary from 350 to 265 mgs., and for the plasma from 280 to 210 mgs. The evident increase at the end of pregnancy may be fairly regarded, it seems to us, as one of the steps taken to insure lactation—a conclusion reached the more confidently since Meigs, Blather-

wick and Cary have shown that the fat in milk is derived largely, if not exclusively, from the lecithin of the blood.

Fetal blood contains less lecithin than does maternal blood. The quantities in the newborn (290-210 mgs. lecithin for the whole blood and 240-210 mgs. lecithin for the plasma) agree closely with the values accepted as normal in adults.

CHOLESTEROL

Of the chemistry and physiology of cholesterol even less is known than concerning lecithin. In certain biological reactions like hemolysis, to be sure, these lipoids have an antagonistic relation. It has also been observed that they are prone to increase or decrease in the blood simultaneously, as though health required the maintenance of their proper balance. Conforming with this principle the increase in cholesterol during pregnancy may prove to be a protective mechanism useful toward modifying the effect of an increase in lecithin which Robertson and Burnett found a powerful stimulant of growth.

In normal women the total cholesterol varies between 200 and 250 mgs. per 100 c.c. of blood; approximately half is free cholesterol, half is made up of cholesterol esters. However, this proportion varies even in health. During pregnancy the cholesterol values at first are not unlike those in normal women, but they gradually increase in the latter months. At the time of labor we find from 190 to 330 mgs. of cholesterol per unit volume of blood. The distribution of free cholesterol and cholesterol esters follows no definite rule, though in general slightly more cholesterol exists in the free form. The esters are more abundant in the plasma than in the corpuscles.

Fetal blood in which the total cholesterol varies from 120 to 200 mgs. per unit volume is not so rich as maternal blood in this lipoid. The difference depends mainly upon the esters which may be of such small quantity as to escape detection unless a large sample of blood is employed for analysis. The negative findings of Slemons and Curtis are thus explained; for, provided sufficient blood is used in the test, esters are always demonstrable in the fetus.

Although the concentration of cholesterol is never the same in mother and fetus, the difference is neither constant nor characteristic. The similarity in the amounts of free cholesterol in the two circulations to which Slemons and Curtis directed attention remains unexplained, and probably will be so until the general physiology of this substance becomes better understood. It is still impossible to say certainly whether cholesterol is a food, a waste-product, or not properly classifiable as a member of either group. In these circumstances, naturally, little will be gained by theorizing in a special field, though it appears unlikely that the placenta is permeable to cholesterol. Such a conclusion has the support of what is known relative to the physical and chemical properties of the substance and also the support of analyses which demonstrate unequal quantities of cholesterol in the blood of the mother and of the fetus.

Our interest in the lipoids began when Autenreith and Funk announced the finding of a hypercholesteremia in eclamptic women, and consequently our experience with cholesterol determinations covers quite a number of years. The records from the study of more than one hundred and fifty pregnancies of which twenty-three were complicated with eclampsia or allied toxæmias are not included in the tables accompanying this report, because determinations of the fat and lecithin were not made, and also because the entire series affords no greater opportunity for valid conclusions than the smaller group of cases studied more intensively. Cholesterol determinations, we find, give no information with regard to the

TABLE I.—WHOLE BLOOD

Case	Source	Fat	Lecithin	Cholesterol			Remarks
				Total	Esters	Free	
1	Mother	1025	295	220	120	100	Normal labor without anæsthesia
	Fetus	740	275	150	20	130	
2	Mother	970	290	220	100	120	do.
	Fetus	725	245	145	25	120	
3	Mother	1000	320	285	125	160	do.
	Fetus	735	275	160	30	130	
4	Mother	855	295	240	130	110	do.
	Fetus	660	240	140	25	115	
5	Mother	765	330	200	115	85	do.
	Fetus	690	285	130	20	110	
6	Mother	920	285	300	145	155	do.
	Fetus	710	270	140	40	100	
7	Mother	820	310	300	110	190	do.
	Fetus	690	270	180	30	150	
8	Mother	885	300	275	105	170	Normal labor with anæsthesia
	Fetus	815	250	165	15	150	
9	Mother	785	310	250	115	135	do.
	Fetus	660	275	170	20	150	
10	Mother	980	330	265	100	165	do.
	Fetus	730	300	165	25	140	
11	Mother	920	340	290	120	170	do.
	Fetus	590	335	150	20	130	
12	Mother	830	320	195	90	105	do.
	Fetus	760	230	145	30	115	
13	Mother	745	285	285	135	150	do.
	Fetus	590	280	130	20	110	
14	Mother	915	265	240	95	145	do.
	Fetus	730	220	200	20	180	
15	Mother	1120	350	280	115	165	do.
	Fetus	790	260	170	20	150	
16	Mother	885	355	255	110	145	do.
	Fetus	675	250	160	35	125	
17	Mother	740	300	310	150	160	do.
	Fetus	645	210	185	25	160	
18	Mother	855	295	240	110	130	do.
	Fetus	735	230	150	10	140	
19	Mother	690	330	250	140	110	do.
	Fetus	500	290	150	20	130	
20	Mother	855	285	270	115	155	Low Forceps
	Fetus	735	240	160	20	140	
21	Mother	880	300	240	100	140	do.
	Fetus	740	260	150	30	120	
22	Mother	920	350	330	205	125	Breech Extraction
	Fetus	815	255	225	20	205	
23	Mother	940	265	220	165	55	Low Forceps
	Fetus	735	240	120	10	110	
24	Mother	805	320	300	145	155	do.
	Fetus	615	290	195	40	155	

approach of eclampsia and assist neither in directing its treatment nor in foretelling the outcome of the disease. While the quantity of cholesterol may be increased in eclampsia, not infrequently even larger quantities are associated with normal pregnancy. On the other hand, the ordinary values may be found in cases of eclampsia. In fact, our experience teaches that there is nothing characteristic in the behavior of the blood cholesterol during the course of this complication of pregnancy.

Summary

1. During the latter part of pregnancy, the fat, lecithin and cholesterol of the blood are more abundant than usual. This change, most likely, represents a preliminary step in the preparation for lactation.

2. The mother's blood contains much more of these substances than the fetal blood does. The difference which exists between the two organisms in this respect varies from case to case; and the values prevailing in one seem to be entirely independent of those in the other.

3. The results of blood analyses in human subjects, like the biological evidence obtained from vital staining experiments in rodents and carnivora, teach that the placenta is impenetrable to fat and lipoids.

4. Fetal fat, then, must be synthesised; probably from glucose, which is freely supplied by the mother in accord with the demands of her offspring.

5. No characteristic change in the blood fat or blood lipoids accompanies the development of eclampsia and allied intoxications of pregnancy.

TABLE II.—PLASMA

Case	Source	Fat	Lecithin	Cholesterol			Remarks
				Total	Esters	Free	
1	Mother	1050	230	215	120	95	Normal labor without anæsthesia
	Fetus	775	220	145	20	125	
2	Mother	990	240	220	100	120	do.
	Fetus	740	235	150	15	135	
3	Mother	1060	240	290	125	165	do.
	Fetus	790	225	160	20	140	
4	Mother	905	235	230	120	110	do.
	Fetus	665	200	130	10	120	
5	Mother	800	230	195	115	80	do.
	Fetus	725	220	125	20	105	
6	Mother	965	240	310	140	170	do.
	Fetus	745	235	145	15	130	
7	Mother	875	255	295	115	180	do.
	Fetus	720	240	185	10	175	
9	Mother	915	230	245	115	130	Normal labor with anæsthesia
	Fetus	825	225	170	20	150	
10	Mother	1035	230	275	130	145	do.
	Fetus	785	220	195	30	165	
12	Mother	900	240	210	100	110	do.
	Fetus	770	230	150	25	125	
13	Mother	790	240	295	135	160	do.
	Fetus	635	240	125	20	105	
14	Mother	945	225	245	100	145	do.
	Fetus	735	215	210	15	195	
15	Mother	1240	270	300	180	120	do.
	Fetus	840	200	180	30	150	
16	Mother	945	280	260	160	100	do.
	Fetus	715	200	170	40	130	
22	Mother	1000	250	330	210	120	Breech Extraction
	Fetus	825	240	230	25	205	
23	Mother	1020	210	230	155	175	Low Forceps
	Fetus	790	190	130	10	120	
24	Mother	860	255	310	150	160	do.
	Fetus	675	230	190	20	170	

NOTE.—The Case Numbers in the above table are the same as those used in Table I. Thus it becomes a simple matter to compare the whole-blood and the plasma values for a given mother and fetus.

TABLE III.—TOXÆMIAS
Whole Blood

Case	Source	Fat	Lecithin	Cholesterol			Remarks
				Total	Esters	Free	
25	Mother	790	300	210	100	110	Pre-eclamptic Toxemia.
	Fetus	655	290	145	40	115	8th Month Pregnancy.
26	Mother	870	380	175	90	85	Pre-eclamptic Toxemia.
	Fetus	685	290	110	10	100	At full term.
27	Mother	890	290	235	110	125	Pre-eclamptic Toxemia.
	Fetus	660	240	150	15	135	8th Month Pregnancy.
28	Mother	870	240	215	120	95	Ante-partum Eclampsia.
	Mother	830	235	210	115	95	After Delivery.
	Fetus	700	260	140	20	120	9th Month Pregnancy.
29	Mother	955	260	190	90	100	Intra-partum Eclampsia.
	Mother	970	250	220	90	130	After Delivery.
	Mother	960	270	220	90	130	2nd day post partum.
	Mother	880	250	270	105	165	13th day post partum.
	Fetus	740	245	145	10	135	At full term.
30	Mother	975	245	180	105	75	Post-partum Eclampsia.
	Mother	875	260	180	110	70	2nd day post partum.
	Mother	825	245	200	110	90	13th day post partum.
31	Mother	890	290	255	130	125	Albuminuria: Twins.
	Fetus (a)	730	230	135	20	115	8th Month Pregnancy.
	Fetus (b)	690	245	140	25	115	8th Month Pregnancy.

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THE BACTERICIDAL ACTION OF ULTRA-VIOLET LIGHT

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The recent revival of interest in the physiological effects of light and the use of light for the treatment of disease has again brought the bactericidal action of light into prominence. Since 1877, when Downes and Blunt [1] discovered that light has the power to kill bacteria, this property of certain radiations has been the subject of many studies, both because of its special interest from the standpoint of disinfection and because its investigation will reveal some of the laws governing the effect of light upon protoplasm. Reviews of the literature have been published recently by Mayer [2] and Clark.[3] These critical summaries, and the review which we have made, show that, although there have been numerous attempts to gain precise knowledge of the bactericidal action of light, many broad phases of general significance remain uncertain.

The studies reported in this paper were begun in an attempt to find out whether various bacteria have specific sensitivities to ultra-violet light of definite wave-length. It became necessary to determine the upper wave-length limit of radiations which are bactericidal, and about which there is still some uncertainty. As this and other phases of the subject are closely related to the absorption spectrum of bacteria, and the effects of temperature and hydrogen-ion concentration, the investigation was extended to include those factors.*

METHOD

The sources of ultra-violet light were the sparks of various metals (iron, zinc, aluminum, cobalt, tungsten, cadmium) made by the passage of a high voltage current between the metal terminals separated about 1 cm. Sunlight was used in some of the experiments. In studying the effect of the radiations emitted from these sources, it is, of course, necessary to use a method by which the rays can be brought to bear directly upon the bacteria, unimpeded or only slightly impeded by media which absorb them. When organisms in a thin layer are held in a fixed position in the path of these rays, it is further possible to observe the effect exerted upon them by light of all the regions of the spectrum within the range of the prism used. This was accomplished by spreading an emulsion of bacteria upon the surface of a thin layer of agar, about 2 mm. thick, which had been allowed to set upon a glass plate. These flat plates measured 3¼x4¼ inches, and fit exactly into the plate-holder of the Hilger spectrograph which was employed. This spectrograph, having quartz lenses and quartz prisms, was capable of focusing a spectrum from 800$\mu\mu$ to 185$\mu\mu$ in a strip 4 inches long. When a plate coated with agar and smeared evenly with bacteria was fitted into the plate-holder of the spectrograph, the spectrum was focused upon the thin layer of organisms. After exposure, the plate was removed and placed in a large Petri-dish and kept in the incubator at 37° C. for 24 hours. The bacteria which survived the radiation, or which were on portions of the plate which did not lie in the spectrum, grew in a more or less opaque layer, outlining the clear areas where the bacteria had been killed. In this way, the organisms served as a sort of photographic plate for the bactericidal rays. Contact prints were made from these preparations (Plate Fig. 1). This method seems to have been used for the first time by Barnard and de Morgan,[4] and more recently by Browning and Russ.[5]

WAVE-LENGTH LIMITS OF BACTERICIDAL ACTION

In 1919, Browning and Russ [5] found that the upper limit of the bactericidal action of light was reached at wave-length 296$\mu\mu$, and that this coincided with the upper limit of the absorption spectrum which they obtained with an emulsion of *B. coli*. They used a tungsten spark and a quartz spectrograph. In our experiments, using the same kind of instruments, we confirmed this, finding that 296$\mu\mu$ was the longest wave-length lethal for bacteria. Nevertheless, we doubted the validity of these results. Many text-books of bacteriology contain photographs of the bactericidal action which sunlight exerts after its passage through the glass of a Petri-dish, which usually absorbs all rays shorter than 350 to 310$\mu\mu$, and because a number of investigators have found that direct sunlight, which contains no rays shorter than 290$\mu\mu$, rapidly kills bacteria. In the spectrograph, on the other hand, the killing of bacteria by light of wave-length 296$\mu\mu$ is accomplished only after an exposure of an hour or more. Even with the zinc spark, which emits strong radiations at 330 to 340$\mu\mu$, it is not possible to record the effect of the three bright lines in that region upon a bacterial film during an exposure of 4 to 6 hours at 24° C., with the spark 2.5 cm. from the wide open slit of the spectro-

* The work was undertaken under the direction of Dr. Van der Lingen, who was Lecturer in Biophysics in the Johns Hopkins Medical School during the winter of 1921-22. After his return to the University of Cape Town, South Africa, to take up his duties as Senior Lecturer in Applied Mathematics, I obtained the data on the absorption spectrum of a bacterial emulsion, the action of the inner ultra-violet light (390-300$\mu\mu$), the temperature coefficient of the bactericidal action of light, and the effect of hydrogen-ion concentration.

—*S. Bayne-Jones.*

graph. A similar effect was noted by Bovie,[6] who interpreted it as an indication that bacteria, which are habituated to sunlight, are much more sensitive to the ultra-violet light which lies beyond the solar spectrum than to the ultra-violet light included in it. As it was thought that the intensity of the radiations in this inner region of the ultra-violet was insufficient in the spectrum from the metal sparks, sunlight was used. An emulsion of a 24-hour culture of *Staphylococcus aureus* was spread on the surface of a plate of nutrient agar (P_h 7.4). The dish was then covered with a cap of tin, having a slit into which various kinds of glass were inserted. The intensity of the sunlight, and of the light after passage through the glass screens, was measured with a Watkins actinometer. The absorption spectra of the screens were photographed. The culture, under these screens, was exposed to sunlight. After exposure, the plate was incubated at 37° C., and the bactericidal effect, when present, was readily shown by the absence of growth. The results are summarized in Table I.

TABLE I.

Upper Limit of Bactericidal Action of Light.

Source of light	Screen	Wave-length transmitted	Intensity factor	Temperature	Time of exposure	Result
Sun	Quartz	to 294 $\mu\mu$	1	32° C	2 hrs.	Killed
Sun	Glass slide	to 310 $\mu\mu$	0.5	36° C	3 hrs.	Killed
Sun	Crook Glass	to 350 $\mu\mu$	0.66	36° C	3 hrs.	Killed
Sun	Blue filter	520 $\mu\mu$ to 360 $\mu\mu$	0.133	36° C	6 hrs.	No effect

From these results it is apparent that the upper wave-length limit of the bactericidal action of sunlight is at 350$\mu\mu$, and that, with the screens used, light of the visible spectrum did not kill bacteria. It will be shown later that the temperature at the time of exposure had no effect upon the upper wave-length limit of the bactericidal action of light. Hence, the result obtained with the spectrograph and metal-sparks does not exhibit the true upper limit of the germicidal action of ultra-violet light.

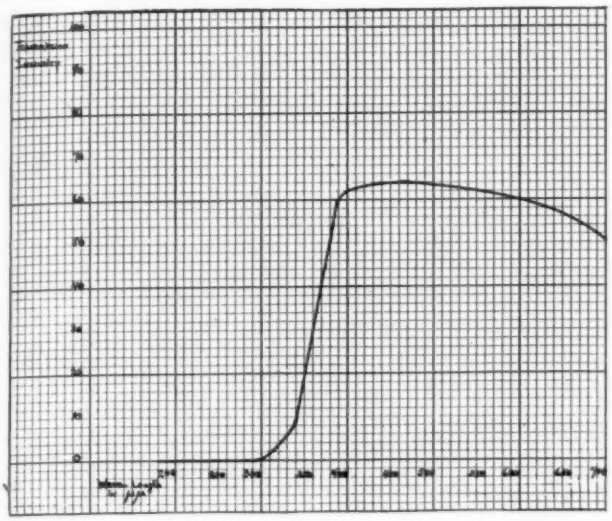
The lower limit of this action may extend below the range of our instrument. The doublet of lines at 185.6$\mu\mu$ in the aluminum spectrum was easily and quickly recorded in the bacterial film. These short radiations are intensely bactericidal. Their effect was to be expected from the work of Hertel,[7] who found that with rays of the same energy, as measured by the thermocouple, the bactericidal effect increased with decreasing wave-length.

Absorption Spectrum of Bacterial Emulsion

In the paper by Browning and Russ,[5] a photograph of the absorption spectrum of a bacterial emulsion is reproduced. This shows a sharply marked absorption band beginning at 296$\mu\mu$ and extending throughout the ultra-violet below this line. The upper limit of the bactericidal action of light, as determined with the tungsten spark and quartz spectrograph, began at 296$\mu\mu$, and hence the germicidal effect of light apparently coincided with the absorption spectrum of the bacteria. This seemed another demonstration of the law of Grotthus, which states that light must be absorbed to produce an effect. In the previous section, however, we have presented evidence that wave-lengths of light as long as 350$\mu\mu$ will kill bacteria if the rays are sufficiently intense. As the law of Grotthus undoubtedly governs this case, it seemed possible that Browning and Russ may have made an incomplete estimation of the absorption spectrum of bacteria. Homogenous emulsions of bacteria, composed of the small bacterial bodies, have a strong scattering effect on a beam of light. In addition, the absorption of light by bacteria may be due to all of the various components of the cells, such as pigments, lipochromes, lipoids, proteins, etc., giving a complex band. That this is the case is shown by the absorption spectra of alcoholic extracts and alkaline solutions of bacteria, which differ somewhat from the absorption spectrum of the whole emulsion. It has not been possible for us to obtain the absorption spectrum of a solution of bacteria in a non-absorbing solvent. Browning and Russ do not state the concentration of the bacterial emulsion which they used, and do not discuss the effect of the scattering of light by the bacterial particles in the emulsion. It can be determined readily that emulsions of bacteria apparently absorb light from the extreme ultra-violet up to 390$\mu\mu$, depending upon the number of bacteria in the emulsion.

The scattering effect of the bacteria in the fluid reduces the intensity of the rays transmitted, but does not alter them qualitatively. With a sufficiently dense emulsion and long exposures, a fairly accurate absorption spectrum of the whole emulsion may be determined. Heavy suspensions of *Staphylococcus aureus*, *Staphylococcus albus*, and *B. coli* in distilled water were used. Each emulsion contained approximately 5 billion organisms per 1 c.c. A tubular absorption chamber 2.5 cm. long and 1 cm. deep, having thin quartz ends, was filled with the bacterial emulsion and placed between the slit of the spectrograph and an iron or zinc spark. A panchromatic plate in the spectrograph was exposed for 10, 15, 20 and 30 minutes to the light which had passed through the emulsion of bacteria. To determine approximately the amount of light absorbed, these plates were compared with photographs of the emission spectra of iron and zinc sparks. To obtain a scale for comparison, the photographs of the spark-spectra were made by serial exposures at different levels on the same panchromatic plate for periods of 1 to 10 seconds. The intensity of the lines registered on the 10-second strip was arbitrarily called 100, and those on the other plates given values in the corresponding lower decades. Then the transmitted lines, registered on the plate made with the light traversing the bacterial emulsion, were compared with the 'scales' of the unabsorbed spark-spectra. The intensity of a line in the absorption spectrum was matched with that of a line of the

same wave-length in one of the spark-spectra, and given a corresponding value. When these values were plotted against wave-lengths, the following curve was obtained. Plate Fig. 2 is a photograph of the absorption spectrum of a bacterial emulsion.



Text—Fig. 1.

Light-Absorption Curve of a Bacterial Emulsion.

The results obtained with the three emulsions of different bacteria and the two sources of light were almost identical. They show that these emulsions completely absorb ultra-violet light up to 300$\mu\mu$, have a strong absorbing action on the wave-lengths up to 350 to 360$\mu\mu$, and transmit 50% of the light at 370$\mu\mu$. The bacterial emulsion transmits, but greatly diminishes, the rays longer than 390$\mu\mu$. From the form of the curve, it is reasonable to suppose that this is due to the scattering effect and is not a true absorption. This determination of the absorption spectrum is more concordant with the law of Grotthus than that found by Browning and Russ. Sunlight of wave-length 350$\mu\mu$ is bactericidal, and bacteria strongly absorb rays of that length. We have not been able to show that light of wave-length longer than 350$\mu\mu$ is bactericidal. From the form of the absorption curve only a slight effect, if any, is to be expected to be exerted by light of the visible spectrum.

Susceptibility of Various Bacteria to Light

Several varieties of Gram-positive, Gram-negative and acid-fast bacteria were spread on agar plates and exposed in the spectrograph to the radiations of various metal sparks, to determine whether any organism was specifically sensitive to the action of particular wave-lengths within the ultra-violet spectrum. The organisms used were *Staphylococcus aureus, Staphylococcus albus, B. coli, B. violaceus, Actinomyces asteroides, B. moelleri* and the so-called *B. lepræ*. Emulsions of equal density of 24-hour cultures were spread on the surface of nutrient agar at P_h 7.4, and exposed at room temperature (20°-25° C.). No significant differences were found. While all varieties were killed by the same radiations, some withstood a slightly longer exposure than others. These differences, however, were not great, and no evidence was obtained that certain bacteria are susceptible to specific lethal wave-lengths.

Effects of Temperature. Temperature Coefficient

The temperature of bacteria at the time when they are exposed to light has been said to increase their sensitivity to the rays of longer wave-length. Thiele and Wolf [8] and Wiesner [9] found that radiations of wave-length longer than 300$\mu\mu$ killed bacteria at 30° to 40° C., and expressed the opinion that, when bacteria at 40° to 48° C. are exposed to light, they may be killed by light of the visible spectrum, including red light. As these experiments were done with bacteria in fluids, the reduction in numbers was estimated by making counts. It seems probable that the temperature alone could have reduced the number of viable organisms in these fluids, as many bacteria are susceptible to heat at temperatures between 40° and 50° C., when exposed to heat for several hours, as they were in these experiments. To test the effect of temperature to the wave-length sensitivity of bacteria, a copper water-jacket was fitted to the back of the glass plate which held the bacterial film on agar. During the time when the organisms were being exposed to radiations in the spectrograph, they were kept at a definite temperature by means of this water-jacket. Under these conditions, a film of *Staphylococcus aureus* on agar, P_h 7.4, was exposed to the spectrum of an iron spark for 2 hours, at 40° C., at room temperature, 24° C., and at 12° to 15° C. In each instance, the bactericidal action of light began at about 296$\mu\mu$, and was never apparent above this line. As has been pointed out, the spectrograph with an iron spark does not bring out the true upper wave-length limit of the bactericidal action of ultra-violet light. Nevertheless, under constant conditions, except for variations in temperature, the limit as determined by these instruments remained constant. The increase in temperature did not render bacteria more sensitive to the longer wave-lengths of light.

Henri and Cernovodeanu [10] have stated that the bactericidal action of light is independent of temperature. At ordinary temperatures, bacteria and some spores can be killed by ultra-violet light in a few seconds. Becquerel,[11] however, found that at the temperature of liquid air, about 6 hours was required to kill anthrax spores and the spores of some fungi with the light from a quartz-mercury lamp. As these investigators used different methods and omit many data from their reports, it is impossible to compare their results closely. But the dif-

ference in the time required for the bactericidal action of light at the two temperatures is so great, that it suggests, at least, that the reaction proceeds more slowly at a very low temperature than at a higher one. As the temperature coefficient of this reaction is of some theoretical interest and has never been determined, experiments were done to discover the effect of temperature upon the velocity of the bactericidal action of light. In all the experiments, a zinc spark was placed 2.5 cm. above the surface of a sterilized cover-glass. Upon the side of the glass facing the spark was placed a drop of a bacterial emulsion in neutral 0.85% NaCl solution. The emulsion contained about 2.5 billion organisms per 1 c.c. Each drop was taken with the same platinum loop, 1 mm. in diameter. On the glass the drops were about 2 mm. in diameter, and less than 0.5 mm. deep. The cover-glass was placed on the flat side of a water-jacket, which permitted raising the temperature above that of the room, was kept at room temperature, or was placed on the flat surface of a piece of ice. The exposures were made at several different temperatures for various periods of time. After the exposure, the drop was taken up by a capillary pipette and transferred to a tube of nutrient broth, which was incubated for 24 hours at 37° C. The shortest exposure at any temperature which killed all of the bacteria in the drop exposed was accepted as indicating the time required for complete bactericidal action of light upon organisms at that temperature. Three series of determinations were made. Exposures for fractions of seconds could not be measured. A summary of the results is shown in Table II.

TABLE II.

Effect of Temperature on Velocity of Bactericidal Action.

Temperature	Time required to kill bacteria			Temp. Coefficient
	Observed	Average	Calculated	
2° C.	28, 28, 27 seconds	27.7		
12° C.			26.1 sec.	1.06
27°.9 C.	23, 24, 24 seconds	23.7		
30° C.	24, 24 seconds	24		
40° C.	22, 24, 23 seconds	23		1.04

The effect of temperature upon the velocity of the action may be calculated for a range of 10° C. from the formula:

$$\text{Temp. Coefficient } (Q_{10}) = \frac{\text{Velocity at } (T+10^\circ \text{ C.})}{\text{Velocity at } T}$$

The velocity of the action may be expressed as the reciprocal of the time required for the radiations to kill the bacteria. Substituting the data given in Table II, the temperature coefficient, then, is found to be 1.06 for the range of temperature from 2° to 12° C., and 1.04 from 30° to 40° C. The average of these values is 1.05.

The order of magnitude of this value is that of the temperature coefficients of most physical and photoelectric effects. It shows that the bactericidal action of light is not entirely independent of temperature, although temperature apparently has only a slight effect upon the velocity of this action.

The data of Table II. may be used also to calculate the constant μ in the formula of Arrhenius.[12]

$$\frac{V_1}{V_0} = e^{\frac{\mu}{2}\left\{\frac{T_1-T_0}{T_0\,T_1}\right\}}$$

where V_1 is the velocity of the reaction at temperature T_1, in absolute degrees; V_0 is the velocity of the reaction at temperature T_0 in absolute degrees; $e=2.7183$; and μ is a constant which is characteristic of the effect of temperature upon a reaction.

Substituting the observed results in the formula, we obtain:

$\mu = 975$, at 27.9° C.
$\mu = 934$, at 40° C.

The agreement of these values is well within the limit of the experimental error. This formula has been used in the study of the effect of temperature upon many types of reactions in bacteriology, and the constant μ has been determined for a number of disinfectants.[12] The value of μ for the bactericidal action of light is much less than that for most chemical reactions, and for such disinfectants as phenol. It must be admitted, however, that the death of bacteria as a result of the action of light may be the end of a complex series of consecutive reactions. As these possible reactions have not been investigated, we hesitate to attach conclusive significance to the coefficients which we have found. It is obvious, however, that the low temperature coefficient, 1.05, and the low value of μ are in agreement with other determinations of these constants for various photoelectric effects.

Effect of Hydrogen-Ion Concentration

Consideration of the possible effect which hydrogen-ion concentration may exert upon the bactericidal action of ultra-violet light has until recently been neglected. The acidity or alkalinity of the medium in which bacteria are placed during the time in which they are exposed to light, with the attendant alteration of the electrical charge upon the bacterial bodies, might have no effect upon the action of light, or might render the organisms more sensitive to the longer rays while increasing their sensitiveness to the ultra-violet radiations. Tests were made to determine whether changes in reaction altered either the previously established wave-length limits or the velocity of the bactericidal action of light.

Bacteria were spread on the surface of nutrient agar, which had the following reactions: P_h 8, 7.4, 6 and 5.5. The preparations were then exposed to the iron spark spectrum for 2 hours, in the manner already described. The upper wave-length limit of the bactericidal action

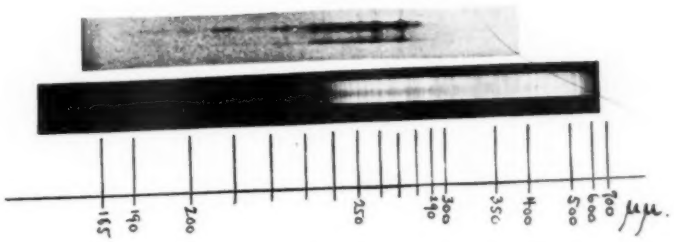

155
190
200
250
300
350
400
500
600
700
μμ.

FIG. I.

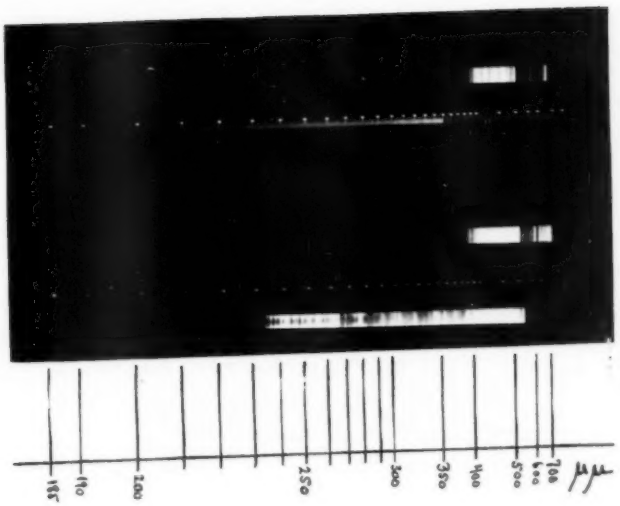

155
190
200
250
300
350
400
500
700
μμ

FIG. II.

was the same on each plate, and was the same as that previously found.

On the other hand, to test the effect of the reaction upon the velocity of the bactericidal effect, emulsions of *Staphylococcus* in alkaline and acid fluids were used. The salt solutions were made with monobasic and dibasic phosphates, adjusted when necessary by the addition of HCl and NaOH, to P_h 9, 8, 7, 6, 4.6, 3 and 2. The reactions were estimated colorimetrically. After the bacterial emulsions at these hydrogen-ion concentrations had been allowed to stand for half an hour, drops were removed with a 1 mm. platinum loop, placed on a sterile cover-glass under the direct radiation from a zinc spark at a distance of 2.5 cm., and illuminated for definite periods of time. After exposure, these drops were transferred by means of a capillary pipette to tubes of nutrient broth at P_h 7.4, and incubated for 24 hours at 37° C. Abundant growth occurred in control transfers taken from the original alkaline and acid mixtures after 1 hour, and in all tubes inoculated with drops of emulsion which had been submitted to sublethal exposure. The temperature during the exposures was 25° C. The results of these experiments are summarized in Table III.

TABLE III.

Relationship of P_h to Velocity of Bactericidal Action.

P_h	Time required to kill bacteria
9	24 seconds
8	22 "
7	22 "
6	22 "
4.6	19 "
3	9 "
2	2 "

These results show that the velocity of the lethal effect of ultra-violet light upon bacteria is greatly accelerated in an acid medium. The acceleration of this action becomes apparent suddenly at P_h 4.6, which is approximately the isoelectric point of the bacteria, as acid agglutination appeared first at this point. In the more acid mixtures the bactericidal effect of light is extremely rapid.

In such a salt solution on the acid side of the isoelectric point the bacteria carry a positive electrical charge. The effect described above is analogous to the action of ultra-violet light upon acid solutions of egg albumin, which has been investigated by Clark.[13] In those experiments she showed that the coagulation of egg albumin by ultra-violet light can be explained on the assumption that it is due to photoelectric action, and from them she has suggested a theory of the physiological effects of light. As bacteria are much more complex than a solution of egg albumin, her theory cannot be applied in detail to this case. The data given in Table III, however, clearly demonstrate the point emphasized by her, that the nature of the electrical charge upon a living organism radiated by ultra-violet light has a direct influence upon the destructive action of that radiation.

SUMMARY AND CONCLUSIONS

This paper describes methods by which exact determinations may be made of the wave-lengths of light which kill bacteria. The sparks of various metals were used as sources of ultra-violet light, except in a few experiments when it was necessary to take advantage of sunlight because of its greater intensity. In most of the work, a quartz spectrograph was used. The absorption spectrum of a bacterial emulsion was correlated with the limits of the bactericidal action of light. The effect of temperature and the influence of hydrogen-ion concentration upon the limits and velocity of the germicidal action of ultra-violet light were determined. From the results of these experiments, the following conclusions are drawn:

1. The bactericidal action of light is confined to the ultra-violet region of the spectrum, beginning at 350$\mu\mu$, and extending with increasing intensity to the shortest wave-lengths measurable with a quartz spectrograph: 185.6$\mu\mu$.

2. These limits coincide with the absorption of ultra-violet light by bacteria.

3. The temperature coefficient for the bactericidal action of light was found to be 1.05 over a range of 10° C., and the constant μ in the formula of Arrhenius was 934 to 975.

4. An increase in hydrogen-ion concentration of the fluid in which bacteria are suspended during their exposure to ultra-violet light increased the velocity of the bactericidal action. The accelerating effect began at P_h 4.6, the isoelectric point, and was very strong in fluids more acid than this.

5. Neither temperature nor the hydrogen-ion concentration rendered bacteria sensitive to the longer wave-lengths of light.

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LEGENDS FOR FIGURES

Text-Fig. 1.—Light-absorption curve of an emulsion of bacteria. Ultra-violet light to wave-length 300$\mu\mu$ is completely absorbed. The absorption of ultra-violet light ends between 350 and 390$\mu\mu$.

Fig. 1.—Contact print of a plate of *Staphylococcus aureus* which had been exposed to the spectrum of an iron spark in a quartz spectrograph. The dark zone in the upper strip shows the region in which the bacteria have been killed. This extends from the extreme ultra-violet to 296$\mu\mu$. Below is printed a photograph of the emission spectrum of an iron spark.

Fig. 2.—Photograph of the absorption spectrum of a bacterial emulsion. The two upper spectra were obtained by exposing the plate to the light from an iron spark transmitted through the bacterial emulsion for 15 and 30 minutes. Below these is printed the spectrum of the iron spark.

THE WASSERMANN REACTION IN THE JOHNS HOPKINS HOSPITAL*

By Albert Keidel, M.D.,
and
Joseph Earle Moore, M.D.
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Since January 1918 the Wassermann Laboratory of the Syphilis Department has examined the sera of about 45000 patients. A survey of the results of these examinations has brought out several points which we feel are of sufficient interest to warrant record. In the first place, the material is of sufficient magnitude to insure accurate statistics. Secondly, the tests have been made with a uniform technique and by the same technical staff throughout, a point which also makes for accuracy. The only previous reports of the incidence of positive Wassermanns in patients of this Hospital have dealt either with special groups of the hospital population,[1, 2] or have included only a small number of cases.[3, 4]

Technic of the Wassermann Reaction in the Department of Syphilis of the Johns Hopkins Hospital:—

1. Series of Wassermann examinations are made daily, so that specimens are usually less than 24 hours old.

2. The patient's serum, removed from the clot just before testing, is diluted with four parts of 0.85 per cent salt solution, and is inactivated in a water-bath (thermostat) at 55°-56° C. for 30 minutes. It is then distributed for examination in test-tubes (12×200 mm.) as shown in Protocol II, Table I.

3. Pooled guinea-pig serum from at least 6 pigs is collected daily, free from cells, after standing overnight with the clot in the ice-box. It is diluted in the proportion of 1 part serum and 9 parts salt solution, and its complement activity determined in terms of known amboceptor activity, as shown in Protocol I, Table I. A daily record of this complement-amboceptor activity is kept, from which a standard for comparison in the selection of suitable sera for complement is established. Actual variations in degree of complement activity occur within very narrow limits when blood from a sufficient number of pigs is pooled. If activation of the amboceptor is accomplished in a sufficiently high dilution, the complement is acceptable; otherwise it is discarded. The prepared complement is kept in the ice-box until used; it is distributed in the tubes of a series of tests as shown in Protocol II.

TABLE I.

Protocol I. Complement titration.* Water-bath incubation 30 minutes.

Scale of amboceptor titration	1-200	1-400	1-800	1-1600	1-3200	1-6400	1-12800
Amboceptor as diluted	.5cc	.5cc	.5cc	.5cc	.5cc	.5cc	.5cc
Complement 1-10	.5cc	.5cc	.5cc	.5cc	.5cc	.5cc	.5cc
Sheep cells 5% suspension	.5cc	.5cc	.5cc	.5cc	.5cc	.5cc	.5cc
NaCl Solution .85%	1.0cc	1.0cc	1.0cc	1.0cc	1.0cc	1.0cc	1.0cc

* Titre is determined by interpolation based on the highest dilution in which hæmolysis is complete, and the degree of hæmolysis in the next highest dilution.

Protocol II. Wassermann Test and Controls.

	Patient's serum 20% dilution	Complement 10% serum	Antigens 10% emulsions Ext. A.	Antigens 10% emulsions Ext. B.	Salt solution		Hæmolytic amboceptor 4 units	Sheep cell suspension 5%
Comp. control Hæmolysis	0	.4cc	0	0	.4cc	Water-bath incubation 1 hr. or less	0	.2cc
Antigen control anticomp.	0	.2cc	.4cc	0	0		.2cc	2cc
Antigen control anticomp.	0	.2cc	0	.4cc	0		.2cc	.2cc
Patient's serum control	.4cc	.2cc	0	0	0		.2cc	.2cc
Test 1	.2cc	.2cc	.2cc	0	0		.2cc	.2cc
Test 2	.2cc	.2cc	0	.2cc	0		.2cc	.2cc

4. Antigens are made from normal, lean, fresh beef hearts by extraction with absolute ethyl alcohol. Twenty-five grams of finely ground heart muscle are extracted by shaking with 100 c.c. of absolute alcohol, and filtered. The filtrate is divided into two lots, one of which is fortified by the addition of 0.2 per cent cholesterol. These are referred to as the cholesterolized (A) and the plain (B) extracts. They are preserved in dark glass bottles at room temperature. They are both employed as 10 per cent emulsions in salt solution, and in this form must not be hemolytic or anticomplementary in twice the quantity employed in the test. Fresh extracts are made not less frequently than once a month, and their antigenic activity is determined by comparison with extracts previously in use. The comparison of new with old extracts is made by a system of parallel titrations in which a fixed amount of the extracts

* This statistical research has been aided by grants from the United States Interdepartmental Social Hygiene Board and the American Social Hygiene Association.

is set up against diminishing amounts of a patient's positive serum. No fresh extract is employed routinely the titre of which is below that of the one previously accepted. The 10 per cent emulsions are made by adding extracts (1 part) slowly to salt solution (9 parts), shaking the mixtures well as the addition proceeds. Fresh emulsions are made daily, and are distributed in the tubes of the series as shown in Protocol II.

5. The antisheep hemolytic system is employed. Hemolytic amboceptor is made in the usual way, and only high titre sera are accepted. This serum is collected under aseptic conditions and is preserved in sealed tubes at ice-box temperature without the addition of a preservative. It is titrated with pooled complement in parallel series with amboceptor of known strength, and its average titre determined in this manner. A fresh 1 per cent solution of amboceptor is made every day. From this solution are made the complement titration (Protocol I), and the total dilution employed in the series. Four units of amboceptor are used and the amount added to the tubes of the series, as is shown in Protocol II.

Sheep's whole blood is obtained at an abattoir twice a week. It is collected in flasks containing 1.5 per cent sodium citrate in 0.85 per cent salt solution, to which is added 0.2 per cent formalin (40 per cent). The cells are freed from serum, citrate and formalin by washing in the usual way, being sedimented in the centrifuge 6 times in this process. The last sedimentation is accomplished at a standard speed for 30 minutes in a graduated tube, the corpuscle mass suspended in 19 parts of salt solution, and distributed as shown in Protocol II.

Tests are carried out in a uniform total volume of 1 c.c., and the preparations of the various ingredients described above are made so that the desired number of units to be added of each is contained in a volume of 0.2 c.c. All routine tests are made by water-bath incubation at 37.5° C.; the ice-box method of fixation being reserved for sera requiring special study. Patients' sera are controlled for anticomplementary substances, complement for hemolysin, and antigen for anticomplementary action on the day of each series. The first incubation is for 30 minutes to allow fixation. After addition of the hemolytic system, the second incubation is continued until all controls have cleared up, but not for longer than one hour.

Results are read immediately after the second incubation and are recorded as follows: 100 per cent fixation—positive; 75 per cent fixation—suggestive positive; 50 per cent fixation—doubtful; 25 per cent fixation—suggestive negative; no fixation—negative.

Wassermann Survey of the House Medical Service:—In the house medical service, including both public and private wards, the Wassermann reaction is a routine for every new patient. Between January 1918 and June 1922, 7154 Wassermann tests have been performed on hospitalized medical patients. The results are shown in Table II. The generally recognized high incidence of syphilis in the colored race is well illustrated. Adequate figures for the incidence of syphilis in patients of the upper social level are very few in the literature. On the basis of our Wassermann results, 7.1 per cent of private male patients were syphilitic, while only 2.4 per cent of the females had syphilis. The figure for the males is probably high for general application, since many of these patients were admitted on our own

TABLE II.

The Wassermann Statistics from the Medical Wards. January 1918—June 1922.

	MALE				FEMALE				TOTAL		
	Negative	Doubtful*	Positive	% Positive	Negative	Doubtful	Positive	% Positive	Total	Negative	% Positive
White private patients	1237	14	81	7.1	1059	8	19	2.4	2418	2296	5.0
White publ. ward patients	1444	22	140	10.0	1073	5	42	4.1	2726	2517	7.6
Colored	644	51	174	25.8	905	54	182	20.0	2010	1549	22.9

* In this and the succeeding tables of this paper, the Wassermanns have been recorded as negative when the result was complete hemolysis, doubtful when hemolysis was 50 per cent or more, and as positive when hemolysis was 25 per cent or less.

service for complaints directly referable to syphilis. This is to some extent offset by the fact that a large number of known syphilitics had negative blood Wassermanns, as is shown by Table III, in which are tabulated the

TABLE III.

Clinical Diagnosis of 4000 Consecutive Medical Discharges. The Johns Hopkins Hospital.

	TOTAL CASES		Non-syphilitic	Probable syphilis	Syphilis	Per cent syphilis	
White	Male	1000	864	8	128	13.6	9.7
	Female	1000	941	8	51	5.9	
Colored	Male	1000	708	24	268	29.2	25.4
	Female	1000	784	13	203	21.6	

discharge diagnoses of 4000 consecutive medical admissions on the public wards. The actual incidence of syphilis in males and females is respectively 3.5 per cent and 2 per cent greater than the incidence of the positive Wassermann.

The Obstetrical Service:—The Wassermann data for this service, made up of presumably healthy women, have recently been studied by Williams in a series of 4000 consecutive admissions. It is unnecessary to do more than to refer to his conclusions. Of 4000 pregnant women, 2.48 per cent of all whites, and 16.29 per cent of all colored patients, gave a positive Wassermann in the blood. An additional 4000 Wassermanns, done on the cord blood of the infants of these women, were positive in only about 1 per cent, showing the unreliability of the test in the new-born.

The Gynecological Service:—The Wassermann is used routinely in hospital cases. In the last 17 months, 839 tests have been performed, as shown in Table IV. It will

TABLE IV.

The Gynecological House Service.
January 1st, 1921—June 1st, 1922.

	White Females				Colored Females			
	Negative	Doubtful	Positive	% positive	Negative	Doubtful	Positive	% positive
Wards (routine)	464	3	16	3.9	263	12	51	19.0

be noted that the percentages for white and colored women are almost precisely the same as those obtained on the medical service.

The Pediatric Service:—In both dispensary and hospital divisions of this service, made up of children under the age of 12, Wassermanns are not performed routinely, but are requested "when there is a history of syphilis or miscarriages and prematurity, in cases with clinical evidence suggesting syphilis; and in most cases of mental retardation, epilepsy, etc." As a matter of fact, tests are performed in only 24.4 per cent of the admissions to this service, so that the incidence of positive Wassermanns, as shown in Table V, is much too high. The

TABLE V.

The Pediatric Service (Harriet Lane Home).
January 1918—June 1922.

	MALES				FEMALES			
	Negative	Doubtful	Positive	% Positive	Negative	Doubtful	Positive	% Positive
	1556	15	187	10.6	1315	10	185	12.2

figures are rendered still less valuable as an estimate of the incidence of syphilis by the fact that an effort is made to examine all the brothers and sisters of every syphilitic child. The table is therefore of interest, chiefly because it shows the very low percentage of doubtful Wassermanns (0.7 per cent) in this class of patients. In other words, the Wassermann is usually strongly positive in congenital syphilis.

The Dispensary Service:—The data for all service of the dispensary, except the Pediatric and Obstetrical Services (given above) and the Department of Syphilis, are given in Table VI. Two time periods are covered, the first from January 1921 to May 1922 inclusive, the second from January 1918 to May 1922 inclusive. The shorter period covering the last 17 months is recorded separately because, owing to more accurate filing, it was possible to divide the cases by sex, though not by race. In none of these dispensary departments was the Wassermann used routinely, Table VII showing for each department the

TABLE VI.

The Dispensary Services.

SERVICE	January 1st, 1920—June 1st, 1922. MALE				FEMALE				Total 1918-22.				
	Negative	Doubtful	Positive	% Positive	Negative	Doubtful	Positive	% Positive	TOTAL	Negative	Doubtful	Positive	% Positive
Medicine	982	12	122	12.0	589	13	79	13.5	4635	3906	127	602	15.7
Gastro-intestinal	337	1	47	12.4	151	3	16	11.1	1030	902	20	108	12.4
Phipps Tuberculosis	95	—	6	5.9	69	1	14	17.8	827	711	26	90	13.7
Neurology	245	5	27	11.5	143	—	23	13.8	1138	965	30	143	15.2
Surgery	107	4	35	26.7	80	3	34	31.6	942	694	28	220	26.3
Gynecology					156	4	36	21.4	1678	1362	42	274	12.8
Genito-urinary	399	10	100	21.6					2008	1552	74	382	22.7
Orthopedics	163	3	28	15.9	76	3	19	22.4	698	560	17	121	19.5
Ophthalmology	102	1	21	17.7	77	1	16	18.0	603	440	27	136	27.0
Laryngology	216	4	27	12.5	181	5	61	26.7	1347	1009	50	288	25.2
Dermatology	134	3	37	22.9	115	1	66	36.8	735	459	24	252	37.5
TOTAL	2780	43	450	15.0	1637	34	364	19.5	15641				

TABLE VII.

The Proportion of Wassermann Tests to New Patients,
January 1921—June 1922.
The Dispensary Services.

Department	Total new cases	Total Wassermanns done	Per cent of new patients on whom Wassermanns were done
Medicine	5264	1797	34.1
Gastro-intestinal	2148	555	21.1
Neurology	1553	443	28.5
Phipps Tuberculosis	1331	185	13.8
Surgery	6071	263	4.3
Orthopedics	2969	292	9.8
Genito-urinary	2475	509	20.5
Laryngology	4721	494	10.4
Dermatology	2165	356	16.4
Ophthalmology	2940	218	7.4
Gynecology	4091	196	4.8
Harriet Lane Home	4956	1211	24.4

percentage of total admissions on whom tests were performed. As might be expected, those services which make the most use of the test, such as the Medical Dispensary, show the lowest incidence of Wassermann positives. Among the clinics which request the test only when the diagnosis of syphilis is directly suggested by history or physical findings, it is interesting to note that Dermatology, Ophthalmology, Surgery, and Laryngology show the highest percentages of syphilis, with Dermatology well in the lead. It is also of interest to note that in practically every department, there is a higher percentage of positive tests in women than in men. This is doubtless due to the fact that the examining physicians are somewhat more suspicious of syphilis in men than in women, and request a test more frequently and on less positive evidence.

Owing to the fact that routine tests were not employed, it is of no importance to subject these results to more detailed analysis.

DISCUSSION

From the practical standpoint, the only essential discussion is one which points to the value of the Wassermann test as a routine procedure. This can best be shown by the application of statistical data to the dispensary services.

It is generally admitted that the aim of medicine is not only curative, but preventive. Either in private or dispensary practice, the physician, no matter what his specialty, should be able not only to examine and treat the patient for the condition of which he complains, but also to perform a general examination sufficiently thorough to detect defects of which the patient may be unaware. In dispensary practice, this type of examination is possible only in those departments directly under the charge of internal medicine, since it cannot be expected that patients reporting for refraction, a lacerated finger, or chronic tonsillitis, for example, can be subjected to the time-consuming procedure of a complete medical survey.

Syphilis, however, is a serious condition which prevails only too commonly and which, in most instances, can be detected by the simple procedure of a blood Wassermann reaction. To secure specimens for the test consumes so little time that it ought to be part of the indispensable routine of all departments. In order to show the advantage of this procedure in preventive medicine, it is of interest to examine the difference between the hypothetical number of cases of syphilis which might have been detected in the various dispensaries, had the Wassermann been routine, and the number actually discovered.

Of 4736 public ward patients, white and colored, on the medical house service (where routine tests were done) 14.2 per cent had positive Wassermanns (7.6 per cent of whites, 22.9 per cent of negroes), as shown in Table I. The social level of these patients corresponds closely to that of dispensary patients, though in the dispensary there are relatively more negroes. This difference in racial distribution would tend to make the routine figures for dispensary patients even higher. Assuming, however, that, had the Wassermann been employed routinely in all departments of the dispensary, 14.2 per cent of the tests would have been positive, we arrive at the data shown in Table VIII. It appears from this table that no department was more than 35 per cent efficient in detecting syphilis among its patients, and that in the dispensary as a whole, in a period of 17 months. 4246 potential syphilitics were allowed to go undetected.* Could these patients be discovered and subjected to adequate treatment, it is absolutely certain that potential sources of infection to others might be eliminated, the birth of many syphilitic babies might be prevented, and the grave late consequences of syphilis, particularly cardio-vascular and neuro-syphilis, might be partially done away with.

TABLE VIII.

A comparison of the actual number of syphilitics found in the Dispensaries in 17 months with the theoretical number which might have been detected by the routine application of the Wassermann; A study in efficiency in preventive medicine.

Department	Total new patients	Potential number of syphilitics *	Actual number positive Wassermanns	Percentage efficiency
Medicine	5264	747	226	30.2
Gastro-intestinal	2148	304	67	22.0
Neurology	1553	220	55	25.0
Phipps Tuberculosis	1331	188	21	11.1
Surgery	6071	862	76	8.8
Orthopedics	2969	431	53	12.2
Genito-urinary	2475	351	110	31.3
Laryngology	4721	670	97	14.4
Dermatology	2165	307	107	34.8
Ophthalmology	2940	417	39	9.3
Gynecology	4091	540	40	7.4
	35728	5137	891	17.3

* See Text.

The statistics tell their own story. In this hospital, and in most others, thousands of patients with syphilis are allowed to pass through without detection or treatment because of the omission of the Wassermann test as a routine. The Wassermann test, while of course not absolutely reliable as an index to syphilitic infection, is,

* Even if the percentage of routine positive Wassermanns in the dispensary class of patients were much lower, for example, 10 per cent instead of 14.2 per cent, the force of the argument would remain unimpaired.

nevertheless, the best available guide and should be a routine procedure on every patient who enters. A general hospital should provide sufficient laboratory space and equipment to make this possible, and should organize and maintain a sufficiently well staffed and organized clinic to permit adequate anti-syphilitic treatment for every patient discovered.

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MINUTE UPON THE DEATH OF DR. WILLIAM STEWART HALSTED

The recent death of William Stewart Halsted, Surgeon-in-Chief of the Johns Hopkins Hospital and Professor of Surgery in the Medical Department of the Johns Hopkins University, has removed from this Board one of its original and most honored members. His memory will always be cherished by both institutions, as he was a potent factor in the organization of the work of the Hospital and Medical School.

Born in New York, September 23, 1852, of a family prominent in business, philanthropic and social life, he received his early education in that city and at Andover Academy, and later graduated in Arts at Yale University. He pursued the study of medicine in the College of Physicians and Surgeons, now a department of Columbia University, and received the degree of Doctor of Medicine in 1877. He further studied in Europe at Vienna, Leipsic and Würzburg in 1878-1880. Returning to his native city, he entered with great energy and success upon the private teaching of surgery and gave instruction to large classes. He was Attending Surgeon to Charity, Bellevue, Presbyterian, Roosevelt and Emigrant Hospitals, and during this period devoted much attention to methods of improving the science and art of surgery.

Later as one of a company of eager and earnest students he came to Baltimore to follow Dr. Welch's classes in pathology at the Johns Hopkins University. Here he made further studies in various problems of surgical technique, especially the healing of wounds and prevention of infection and laid the foundation of lasting surgical success. In 1889, upon the opening of the Johns Hopkins Hospital, he was placed in charge of its surgery, and in 1893, upon the opening of the Medical School, he was made Professor of Surgery, positions which he filled with great honor during the remainder of his life. His breadth of training and indefatigable industry fitted him preeminently for these positions. His most prominent characteristic, perhaps, was thoroughness. Whatever he attempted was conscientiously done in the most scientific and efficient manner, according to the soundest principles of surgery. He combined profound knowledge and unusual experience with great intellectual honesty. There were few branches of surgery which he did not enrich by careful thought and scientific research. He gave special attention to the surgical principles underlying the healing of wounds, the radical cure of hernia and breast cancer, the suture of the intestine, the treatment of aneurysm and the diseases of the thyroid and parathyroid glands. Among his many contributions to surgical science is the discovery of block anæsthesia, which has proved of great advantage both in surgical and dental practice. He was constantly developing new surgical technique and more efficient methods of procedure. He was responsible for the use of rubber gloves in surgery. His innate modesty rendered it difficult for him to claim the recognition which really belonged to him. One of his most useful contributions to surgery was his careful, thorough and systematic training of a school of younger surgeons in scientific work and his rigid insistence upon the rule that all surgery which came from him, or passed under his notice, or was performed by his assistants, should be thoroughly, conscientiously and efficiently done.

He received many titles and honorary degrees both at home and abroad.

He was a truly great surgeon and his work at the Johns Hopkins Hospital marked a new era in the development of American Surgery. His memory will always be cherished by his associates and friends because of his habitual courtesy and kindness and his whole-hearted devotion to his professional work. A great and good man has been removed by death, and all who were associated with him deeply deplore his loss.

STUDIES ON SUGAR TOLERANCE

By RALPH H. MAJOR

(From the University of Kansas School of Medicine, Kansas City, Kansas)

Increasing interest in the fate of carbohydrates in health and disease is shown by the reports from many clinics. The work of Tachau,[22] Jacobsen,[13] Hopkins,[12] Hamman and Hirschman,[10, 11] and Janney and Isaacson,[15] has simplified the study of certain phases of carbohydrate metabolism so that the glucose test, as devised by the last-named authors, has come into wide use.

A review of the recent literature confirms the great value of this test in the diagnosis of diabetes mellitus, and of certain other diseases. In any disease in which there may be a disturbance of carbohydrate metabolism, the application of the glucose tolerance test gives information of great interest and often of great value. The accumulation of all available data in regard to the application of the test is highly desirable, and promises to shed light on our knowledge of the retention, utilization, and excretion of carbohydrates.

Differences of opinion exist in regard to the ability of the kidney to hold back sugar until a definite blood sugar concentration is reached. Benedict,[3] for example, states that normal individuals are constantly secreting sugar in the urine, and suggests that the difference between diabetics and normal individuals is a quantitative one. This view is opposed by Folin [5] who says: "The concept comprised in the term glucose threshold is something not only approximately true; the concept is absolutely correct. Hyperglycemia definitely below the threshold does not normally produce the slightest leakage of glucose through the kidneys, and normally not a trace of absorbed or circulating glucose is lost."

The following report is presented as an addition to the data already accumulated relating to the effect of carbohydrate ingestion upon the blood sugar and upon urinary glucose in health and disease. As the individuals studied all received pure glucose, no attempt was made to study the "glycuresis" which Benedict noted after an ordinary carbohydrate meal, and which, according to Folin, is not normally obtained from the ingestion of glucose, but is due to "foreign unusable carbohydrate materials," and "decomposition products due to cooking, canning, and baking."

Some of these observations were made at the Henry Ford Hospital, Detroit, and the remainder in the medical service of the Bell Hospital, Kansas City.

METHODS

The earlier tests were carried out according to the directions of Hamman and Hirschman,* 100 gm. of glucose being given on an empty stomach. Later, the patients were given 1.5 gm. of glucose per kilogram of body weight, this figure being arbitrarily adopted as representing the ingestion of 100 gm. of glucose by an individual weighing 67 kilos (150 lbs.). One half this amount was frequently employed when the test was carried out on diabetics. A few tests were carried out according to the method of Janney and Isaacson,[15] 1.75 gm. of glucose per kilogram of body weight being given. The glucose was given in lemonade containing the juice from one lemon, and approximately 2.5 c.c. of water per gram of glucose. No essential differences were noted in the results obtained by these three methods.

Both blood and urine were examined on a fasting stomach; the glucose was then administered, and samples of blood and urine were obtained every hour over a period extending from four to six hours, occasionally shortened to two hours. In some instances collections were made every half hour. The method of Folin and Wu was used for determining the amount of blood sugar, and for the urinary examinations the method of Benedict and Williams was employed. Urines showing no reaction with Benedict's quantitative solution were not examined further.

NORMAL CONTROLS

The results of this test were studied in eight normal individuals. The lowest fasting value for the blood sugar was 70 mg. per 100 c.c.; the highest value following the ingestion of glucose was 153 mg. per 100 c.c. There was a prompt return to the normal blood sugar level within two hours and no glycosuria was detected. These findings are substantially in agreement with those of Hamman and Hirschman,** Janney and Isaacson,† and Goto and Kuno.[9]

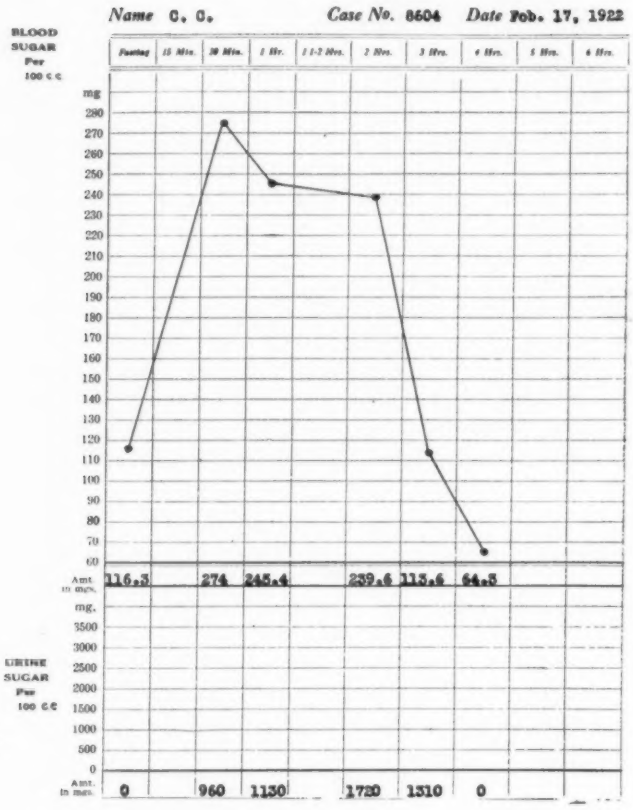
DIABETES MELLITUS

This group of diabetics comprises eighteen cases, ten of which were mild, the other eight proving to be moderately severe. Some of these patients were on a restricted diet, others had had no treatment at the time the test was carried out. The general type of blood sugar curve was similar in all these cases. There was a constant hyperglycemia persisting longer than two hours and accompanied by glycosuria.

* *l. c.*

** *l. c.*

† *l. c.*



155.5 gms. Glucose taken.
97 Kilos. Patient's weight.
1.5 gms. Glucose per Kilo body weight.
Diagnosis: Diabetes mellitus.
Remarks: Mild.

CHART 1.—Example of mild diabetes.

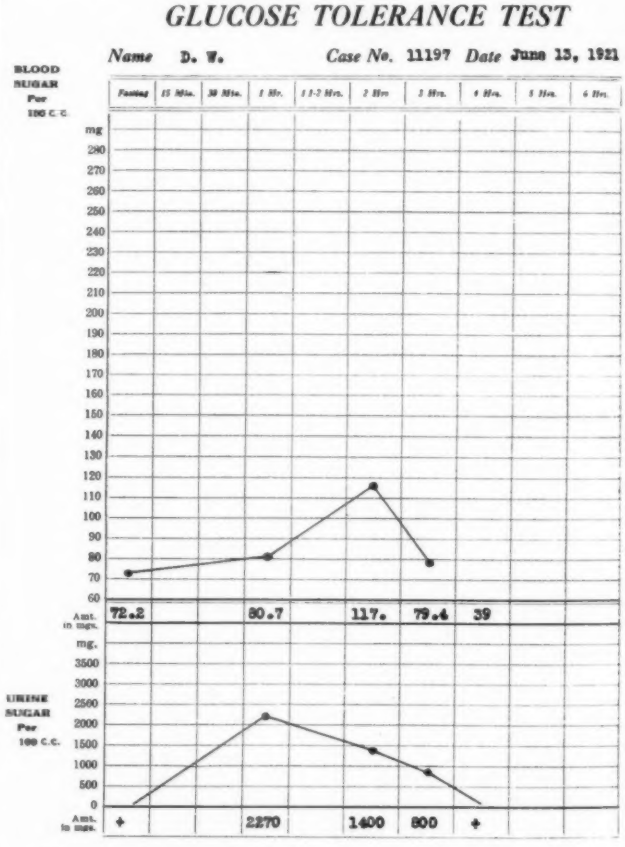
The curves, when plotted, showed some differences in appearance, due largely to differences in the fasting level of the blood sugar, which, when low, gave a strikingly sharp rise following the ingestion of glucose. The height or abruptness in the rise of the curve was not so good as an index of the severity of the disease as the persistence of the "diabetic plateau."

A study of the curves showed considerable variations in the threshold for sugar. Five patients showed no glycosuria with blood sugar levels at 183 mg., 196 mg., 230 mg., and 267 mg. per 100 c.c., respectively. The ages of these patients were fifty, fifty-seven, forty, forty-two, and sixty-four, respectively. The patient showing no glycosuria with a blood sugar of 230 mg. showed 1.1% sugar in the urine with a blood sugar of 242 mg. and proved to be suffering from a mild diabetes mellitus. The patient with a threshold above 196 mg. had a moderately severe case.

Two patients showed threshold values lower than those usually accepted as normal (160-175 mg. per 100 c.c.). One patient showed .18% of sugar in the urine with a blood sugar of 137 mg., and another patient showed a positive urinary sugar reaction with 87.4 mg. of blood sugar, and .7% of urinary sugar with a blood sugar level of 148 mg. Both of these patients were mild diabetics, aged twenty-five and forty-three, respectively, restricted to a low carbohydrate diet, any infraction of which was promptly followed by glycosuria.

Renal Diabetes

Four examples of this interesting condition were encountered. This term as Lewis and Mosenthal [17] emphasize, is a misnomer, and the expression "renal glycosuria" is more accurate. These cases were similar to those reported by Lewis and Mosenthal ‡ and Bailey,[1] and showed a fairly constant glycosuria unchanged by the carbohydrate content of the food. All showed a glycosuria with a normal blood sugar level of 86, 54, 72, and 96 mg., respectively (Chart 2). None had any symptoms or any other urinary findings suggestive of diabetes mellitus. The glycosuria was discovered accidentally, and the



111 gms. Glucose taken.
74 Kilos. Patient's weight.
1.5 gms. Glucose per Kilo body weight.
Diagnosis: Renal Diabetes.

CHART 2.—Renal diabetes. Glycosuria without hyperglycemia.

‡ *l. c.*

patients came to the hospital for the purpose of having their condition studied further.

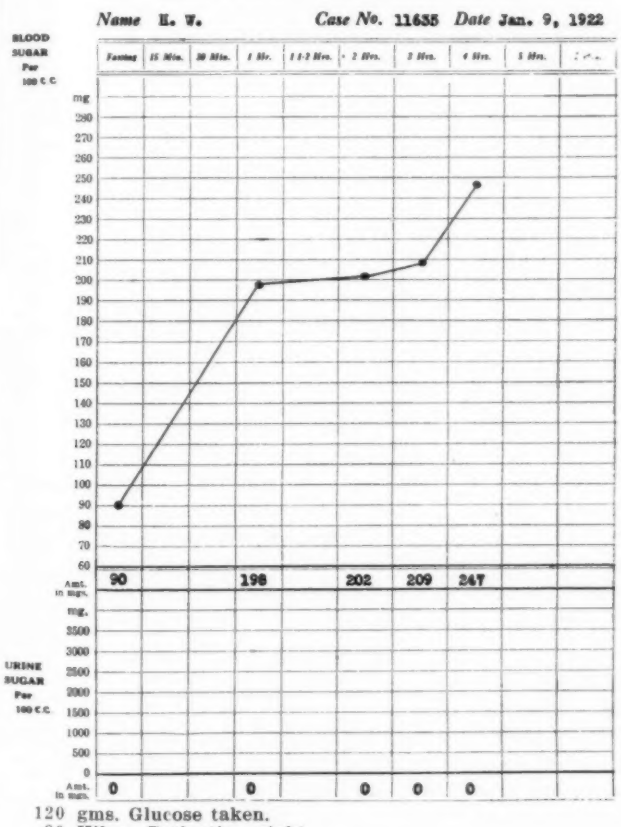
More extended use of the glucose tolerance test will probably bring to light more of these cases. In the past this condition has been considered rare, and Goto,[8] in 1918, collected only twelve cases from literature.

Nephritis

Two cases of chronic nephritis were studied. One patient gave a normal reaction, the other showed glycosuria and hyperglycemia, but with a blood sugar curve falling to normal in two hours. Two patients with arterial hypertension lacking evidence of renal insufficiency showed normal curves. Further studies on such patients similar to those of O'Hare[19] would be valuable.

Four cases of acute nephritis were studied, and all showed similar curves. There was a hyperglycemia persisting for four hours, but no glycosuria save in the three hour specimen of one patient. Three cases were fatal, while the fourth patient recovered.

The highest value for blood sugar was 408 mg., one hour after ingestion. One of the typical curves is reproduced (Chart 3). The curves suggest those obtained by Hamman and Hirschman in their Case 16, but are more striking in degree. The ages of the patients were eleven, forty, thirty-three, and thirty-eight years.

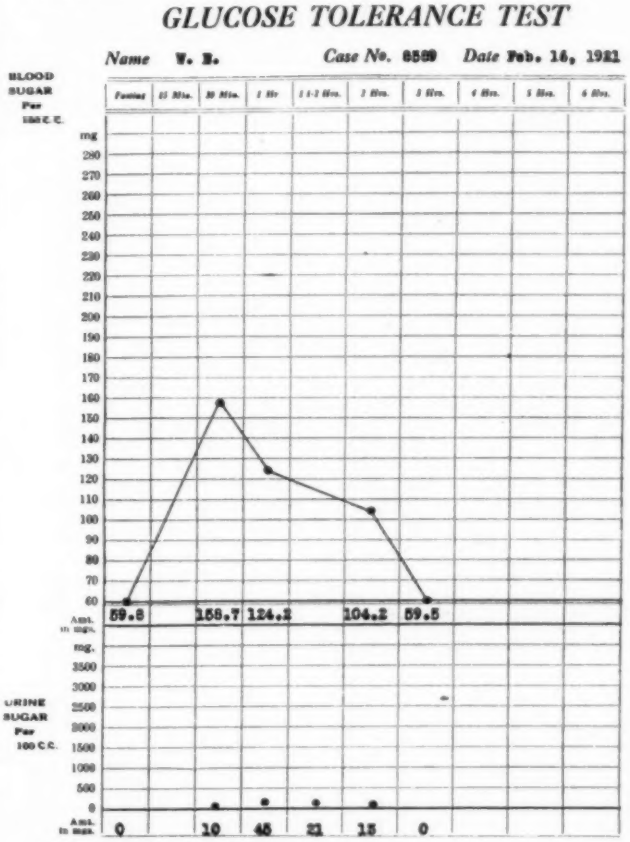


120 gms. Glucose taken.
80 Kilos. Patient's weight.
1.5 gms. Glucose per Kilo body weight.
Diagnosis: Nephritis.
Remarks: Acute.

CHART 3.—Acute nephritis, hyperglycemia, but no glycosuria.

Thyroid Disease

One patient with an outspoken myxedema was studied. This man had on one occasion a fasting blood sugar of 152 mg., which after the ingestion of 1.5 gm. of glucose per kilogram of body weight rose to 169 mg. in half an hour, 163 mg. in one hour, and fell to 110 mg. in two hours. A second test, carried out the following day with three grams of glucose per kilogram, showed a rise from 106 to 233 mg. in half an hour, falling to 120 mg. in two hours. No glycosuria was present during either test.

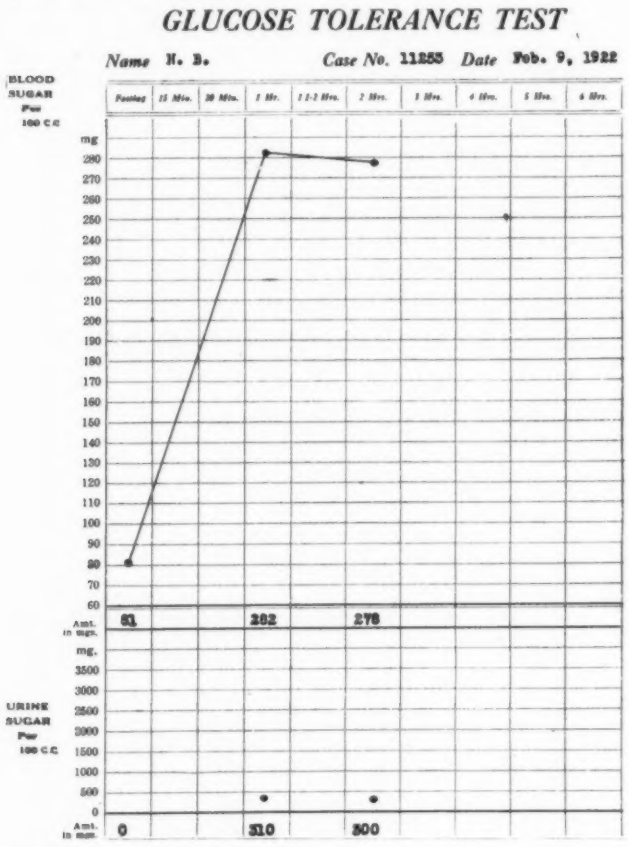


67.5 gms. Glucose taken.
45 Kilos. Patient's weight.
1.5 gms. Glucose per Kilo body weight.
Diagnosis: Hyperthyroidism.

CHART 4.—Hyperthyroidism with lowered renal threshold.

Eight cases of hyperthyroidism were studied, three types of curves being encountered. Four patients showed normal curves, two a glycosuria with a normal blood sugar curve not exceeding 165 mg. (Chart 4), and two typical diabetic curves. The degree of severity of the disease apparently bore no relationship to the type of curve obtained. One of the most severe cases of hyper-

thyroidism showed a normal curve, while another of the same degree of severity showed the interesting curve presented in Chart 5.



96 gms. Glucose taken.
65 Kilos. Patient's weight.
1.5 gms. Glucose per Kilo body weight.
Diagnosis: Graves' Disease.
Remarks: Before operation.

CHART 5.—Diabetic type of curve before operation.

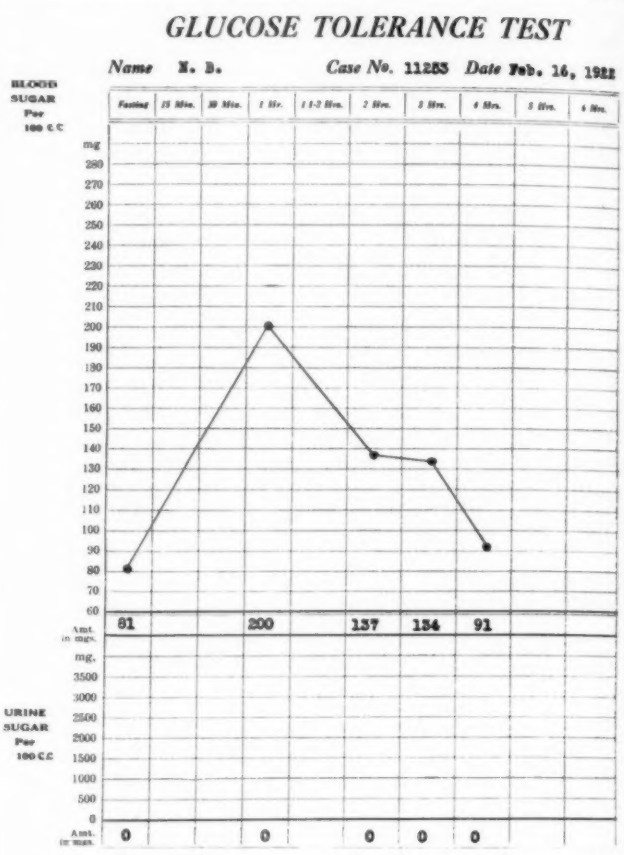
Only one patient had a hyperglycemia on a fasting stomach. (206 mg. per 100 c.c.)

The findings in this group of cases are not in harmony with those of Gayelin,[7] but agree with the observations of Denis and Aub [4] in regard to the rarity of hyperglycemia in hyperthyroidism. These results indicate that a single characteristic curve for hyperthyroidism does not exist. A similar conviction is expressed by Janney and Henderson.[14]

PITUITARY DISEASE

This group consisted of ten cases.

A case of acromegaly showed an increased carbohydrate tolerance. The patient, whose fasting blood sugar was 78 mg., received 3 gm. per kilo; there was no glycosuria, and the blood sugar rose only to 106 mg.



96 gms. Glucose taken.
65 Kilos. Patient's weight.
1.5 gms. Glucose per Kilo body weight.
Diagnosis: Graves' Disease.
Remarks: After operation.

CHART 6.—Curve on same patient as in Chart 5 after operation.

A second test three days later showed practically an identical curve.

Eight patients with marked obesity and other symptoms of Fröhlich's syndrome were studied. All showed a carbohydrate tolerance in excess of 3 gm. per kilogram of body weight. The blood sugar curves, however, showed marked variations. One type was normal, the other type, observed in four patients, showed a marked hyperglycemia (200—320 mg.) which persisted three and four hours, but with no glycosuria.

A patient with diabetes insipidus showed a fasting specimen of 138 mg. and a blood sugar value of 157 at the end of four hours.

These tests indicate an increased carbohydrate tolerance in pituitary diseases often accompanied by a high renal threshold for glucose, when the glucose is administered by mouth. This increased tolerance is not due, however, to a retarded absorption from the bowel, as Wilder and Sansum [22] suggested, since the blood sugar curves clearly prove rapid absorption.

These curves show an abnormal response to the glucose tolerance test, although the type of response is not uniform. A comparison of results in pituitary diseases with those obtained in hyperthyroidism shows no constant type of curve, which is in agreement with the conclusion of Janney and Henderson that "an abnormal blood sugar tolerance curve is to be regarded solely as a general indication of endocrine disturbance."

CANCER

The blood sugar in cancer has been studied by Benedict and Lewis,[2] by Friedenwald and Grove,[6] and by Spence.[21] Benedict and Lewis examined the blood of fifty patients, three hours or more after meals, and found a hyperglycemia in 49%. Friedenwald and Grove, who carried out glucose tolerance tests with 100 gm. of glucose, found a persisting hyperglycemia in 72 out of 75 cases of cancer of the gastro-intestinal tract. Spence studied only four cases, one alone giving a delayed hyperglycemic curve. He points out the influence of age upon the type of blood sugar curve, a factor which has been perhaps too much neglected. Spence states that hyperglycemia with delayed return to normal is frequent with patients over sixty.

We have studied about four cases of carcinoma of the gastro-intestinal tract, two of gastric cancer, and two cases of cancer of the pancreas. All showed a hyperglycemia persisting longer than two hours with no glycosuria.

ULCER

This test was carried out on four patients with indolent leg ulcers. One patient showed a normal curve, the other three a persisting hyperglycemia with a trace of sugar in the urine. These three patients were placed on a diet restricted in carbohydrates and the ulcers disappeared. It is suggestive that they may have been examples of the pre-diabetic stage which has been studied especially by Sherrill.[20]

CONCLUSION

The results of glucose tolerance tests on sixty patients are reported.

The diabetic type of sugar curve with glycosuria is constant in diabetic mellitus. The level of the sugar threshold varied greatly, showing a tendency to be higher in older individuals.

All the cases of acute nephritis studied showed a diabetic type of blood sugar curve usually without glycosuria.

No constant type of curve was obtained in thyroid or pituitary disease. Abnormal curves are frequent and indicate a disturbance of carbohydrate metabolism. Increased tolerance to carbohydrates was found in pituitary disease, often with a high sugar threshold.

Some of the glycosurias encountered in hyperthyroidism are due to a lowered renal threshold.

These observations indicate that as a rule the urine does not show sugar in appreciable amounts until a definite blood sugar level is reached. This level or "renal threshold" varies somewhat in health, but markedly in disease. Repeated tests on the same individuals show marked variations in this "renal threshold." Four individuals classed as "renal diabetics" showed glycosuria with a blood sugar at normal or subnormal levels.

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THE CAUSE OF CERTAIN ACUTE SYMPTOMS FOLLOWING GASTRO-ENTEROSTOMY

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During the past ten years many facts have been added to our knowledge of intestinal obstruction. As the result of experimental studies by numerous workers, it has been clearly proved that from the intestinal contents in cases of obstruction a poison, responsible for the intoxication, can be isolated. The source and nature of the toxic substance or substances are yet a matter of controversy. Whipple [1] and his co-workers think that a proteose formed in the mucosa of the intestine is the toxic factor; others, notably Dragstedt [2] and Davis and Stone,[3] hold that the injurious substances are putrefaction products formed by proteolytic bacterial activity. Sweet and his co-workers [4, 5] have shown that a poison which seems to be identical with that obtained from an obstructed gut in high intestinal obstruction may be isolated from the intestinal content in conditions other than actual obstruction, such as the intravenous injection of high obstruction toxin into normal animals, the removal of the adrenals and in experimental acute fulminating non-bacterial peritonitis. They think that the poison is formed in the cells of the mucosa of the small intestine and that it may be called forth by any of the various conditions cited. They conclude that the poison is not a proteose.

The intravenous injection of material obtained from the lumen above an obstruction or from a closed loop of intestine reproduces the salivation, vomiting, diarrhea, prostation, low temperature, and low blood pressure as seen clinically or in animals suffering from an actual obstruction. The poison which on injection causes such severe symptoms, is, however, entirely harmless when introduced directly into the intestine.

Chemical studies have shed much light on the fundamental nature of the intoxication. In most instances there is a marked rise in the non-protein nitrogenous bodies of the blood. The total non-protein nitrogen may reach 400 mgm. per 100 c.c. of blood. Simultaneously there is a marked increase in nitrogen excretion by the kidneys. These facts show that the toxic agents cause a great destruction of body protein. Although there may be some depression of kidney function as a result of the general intoxication, renal insufficiency has little to do with the high level of nitrogenous bodies in the blood.

Whipple [6] has also shown by the following experiment that an intoxication may develop under conditions similar to those which may follow a gastroenterostomy in the human being. A complete obstruction of the duodenum of a dog was effected at its lower end by section and inversion of the intestine, and the jejunum was joined to the stomach by gastroenterostomy, thus allowing fluid from the stomach to escape into the small intestine. In this animal a definite intoxication supervened, due, he thinks, to absorption from the blind duodenum.

We have been interested recently in the study of three patients in whom rather severe reactions occurred following gastroenterostomy. The symptoms and chemical findings in the blood and urine were characteristic of intestinal obstruction, although there was no reason to suspect an actual interruption of continuity below the gastroenterostomy stoma. The symptoms alone were such as might have been interpreted as due to any one of a number of causes. The chemical studies, however, showed that there was a definite intoxication characterized by marked tissue destruction.

These patients have emphasized to us the importance of an upper intestinal tract intoxication as the cause of certain reactions following operation. We believe that we must attribute the symptoms in these cases to the absorption of a poison probably formed in the duodenum following a gastroenterostomy. The mechanical factor has not been determined, but it is probably an obstruction to the outflow of the duodenal contents.

The case histories and chemical findings are as follows:

CASE 1.

E. C. (Case No. 8,540), admitted to the Henry Ford Hospital, February 1st, 1921, complaining of vomiting, nervousness and rectal trouble. For twenty years she had had attacks of stomach trouble characterized by eructations, gas in the stomach after meals, "cutting and gnawing" pains radiating to the right and left back and chest, and vomiting. She had had several such attacks annually which lasted from two to three weeks. She also complained of frequent urination associated with some pain. Her personal history was otherwise negative.

On admission the patient was restless and irritable and evidently ill. The general physical examination was negative. the blood pressure was 165/100. The blood count showed 4,576,000 red blood cells, 6,700 leucocytes and 94 per cent hemoglobin. The specific gravity of the urine was 1.007. Tests for sugar and albumin were negative. There was a trace of acetone. The microscopic examination was negative. The patient vomited several times on the day of admission and on succeeding days. Her blood pressure dropped to 140/80 and remained so until operation. The urine showed a trace of albumin at times in subsequent examinations. A concentration test showed a night urine of 162 c.c., a day urine of 543 c.c., and a variation in specific gravity from 1.013 to 1.028. The phenolsulphonephthalein test showed an excretion of 63 per cent in two hours. The blood chemistry was normal. The Wassermann was negative. The X-Ray examination of the gastrointestinal tract showed a hypertonic stomach and a strikingly deformed duodenal bulb. The deformity took the form of two constrictions which divided the barium in the bulb into three segments. The bulb was displaced to the right and fixed in position.

An operation was performed February 18th, 1921. A marked hypertrophy of the pylorus with pyloric stenosis was found. A posterior gastroenterostomy was done. The patient had marked nausea and vomiting, but these symptoms quickly subsided. Three days after operation the phenolsulphonephthalein excretion was 55% in two hours. Seven days after the operation the patient had a "stormy" night with nausea and vomiting. Stomach lavage gave no relief. It was noted that the lavage fluid was not well returned. Her condition grew steadily worse. Twenty-one days after operation she was stuporous, had sighing respirations, and was poorly oriented. The next day she seemed moribund. The blood pressure was 105/70. Soon after, improvement began and recovery gradually took place.

A fluoroscopic examination of the gastrointestinal tract, eighteen hours after operation, when the patient's symptoms were most marked, showed that there was no obstruction, the barium passing satisfactorily through the enterostomy opening. Some barium passed through the pylorus, but the duodenal bulb could not be filled out.

There was no fever or tachycardia at any time. The urine showed a moderate quantity of albumin, hyaline and granular casts and pus cells following operation.

Two hundred and fifty cubic centimeters of six per cent sodium bicarbonate solution were given per rectum four times a day for three days beginning eight days after operation. A similar quantity was given nineteen days after operation for one day only. There was no other alkali therapy. It is not known whether the patient had been taking an alkali before admission.

The patient was last seen July 7th and at that time was entirely free from symptoms.

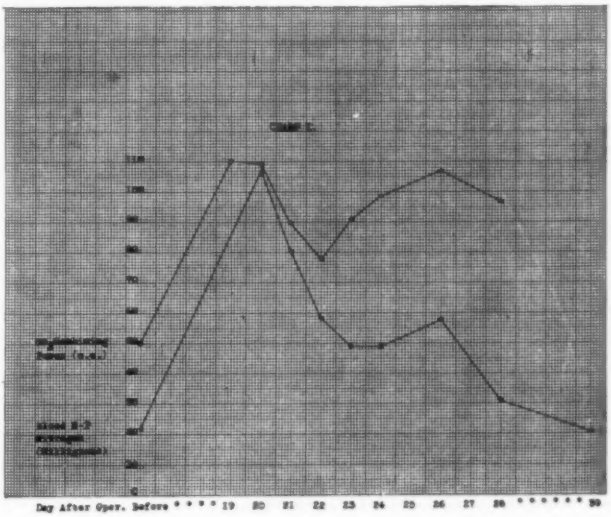
The blood chemical findings are shown in Table I and Chart I.

TABLE I.

Blood Chemical Findings in Case I.

Day after operation	Before	19	20	21	22	23	24	25	26	27	28	***	39
Non-protein Nitrogen	22		109	80	57	48	48		57		31		22
Urea Nitrogen	9		72	47	30	28	31		24		16		28
Sugar	110		143	93	79	108	150		121		86		102
Uric Acid	2.7		7.2	7.1	5.0	5.2	5.0		1.8		2.0		3.5
Creatinine	1.3		2.2	2.7	2.1	2.2	1.8		1.7		1.4		1.5
Creatine	5.7		8.3	6.0	6.5	6.5	7.8						
CO_2 Combining Power	50	110	109	90	76	91	99		107		95		
Chlorides (Plasma)	513		305								440		

Unfortunately the true cause of the patient's condition was not recognized until late; hence no chemical studies on the urine were made.



CASE 2.

L. B. (case No. 11,583), white, male, age thirty-four, admitted to the Bell Memorial Hospital on the service of Dr. M. T. Sudler, December 16th, 1921, complaining of heavy dull pain in the right hypochrondrium. His past history was negative. He dated the onset of his illness as seven months previous to admission, when he began to have soreness below the right costal margin. The symptoms bore no relation to food. He had no nausea, no vomiting, no evidence of gastric or intestinal bleeding. His general physical examination was negative. Urinalysis was negative. The blood examination showed a slight anemia with a normal leucocyte count. Fractional gastric analysis showed an average of 35 acidity per cent of free HCl and 42 acidity per cent of total acid. The X-Ray examination revealed a definite irregularity of the duodenal cap, which was interpreted as being caused by gall-bladder disease or ulcer. His temperature and pulse were normal.

He was put on a Sippy diet for fifteen days and given bismuth subnitrate and sodium bicarbonate without improvement. Operation was advised. This was done January 18, 1921.

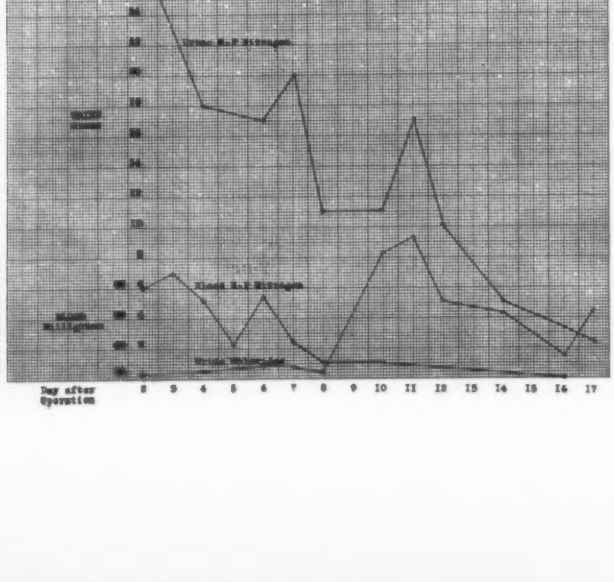
At operation a large indurated ulcer was found on the lesser curvature of the stomach. A gastroenterostomy was done by the no-loop method about three and one-half inches below the duodenojejunal junction. Immediately after the operation the patient was given 1,000 c.c. of saline solution beneath the skin. The day following the operation the patient vomited large quantities of liquid. His temperature was 101°, pulse 88 and respiration 22. The vomiting continued. On the second day the temperature was 102°, pulse 116, and respiration 24. He was quite restless, thirsty, and unable to sleep, and at times definitely cyanotic. He was evidently very ill. The vomitus had an offensive odor. He complained of pain in the epigastrium. Gastric lavage removed liquid dark in color with a foul odor. The third day his temperature was 100.4°, pulse 112, and he appeared to be better. On the fourth day after operation he showed marked improvement and following that his recovery was uneventful.

The chemical findings in the blood and urine are shown in Table II and Chart 2.

TABLE II.

Chemical Findings in Blood and Urine of Case 2.

	Day after operation	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
BLOOD (Mgms. per 100 c.c.)	Non-protein Nitrogen	60	65	56	41	57	42	35		35						30	
	Urea Nitrogen	39	32	10	18	19		7		15						12	
	Sugar	91	135		83	100	137	167		200						134	
	Uric Acid		2.2	2.8	4.6	5.3	3.2	2.6		1.7						2.5	
	Creatinine		2.0	1.8	1.6	1.7	1.3	1.9		1.8						1.8	
	CO_2 Combining Power			67	69	47		54		54						57	
URINE (Grams per Day)	Chlorides (Whole blood)	440			350	400	430	490		390						540	
	Nitrogen	28	6.4+	18		17	20	11	5.8	11	17	10		5.5		3.3	2.5
	Chlorides	0.4	0.1	0.5		0.9	0.9	0.4		8.3	9.4	5.1		1.3		1.4	5.3



CASE 3.

L. T., (Case 11, 646), a farmer, age thirty-one, admitted to the Bell Memorial Hospital, Janury 9, 1922, complaining of stomach trouble. He gave a history of stomach symptoms for thirty years. On admission he complained of pains occurring two hours after meals, sour eructations, disturbed sleep, loss of weight and constipation. On examination the patient did not appear ill. He was under-nourished, had marked pyorrhea, and a slightly enlarged thyroid. The blood pressure was 110/80. A routine examination of the urine was negative. A blood count showed a slight anemia with normal leucocyte count. A stool examination showed no occult blood. The X-Ray study showed an irregularity of the duodenal cap. Operation was advised because of the long duration of the condition unrelieved by medical treatment.

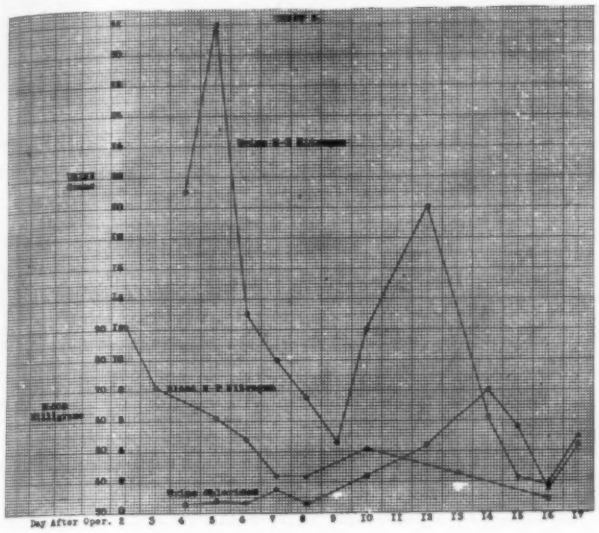
An operation was done January 18th, 1922. A small scar was found in the duodenal wall just below the pyloric sphincter. A gastroenterostomy was done by the no-loop method at a point about three and one-half inches below the duodenojejunal junction. The appendix contained a fecal concretion and was removed. On the day following the operation the patient complained of sharp pain in the abdomen. There was no nausea. Salt solution was given by hypodermoclysis. A blood count showed 24,000 W.B.C. with 88% polynuclears. The temperature was 99.6°, pulse 104 and respiration 20. In the afternoon the temperature rose to 102. On the second day after operation the patient was quite ill and slightly delirious. His temperature was 101.2°, pulse 110. He still complained of abdominal pain. Glucose and sodium bicarbonate were given by protoclysis and 200 c.c. of 25% glucose were given intravenously. On the third day the patient was somewhat improved. Broth was taken in small quantities. His urine was alkaline. On the fourth day liquids were well taken. He rested well without morphine. A part of the day he was somewhat delirious and had delusions. His temperature was 100°, pulse 92, respiration 20. After this his temperature became normal. His further convalescence was uneventful. He did not vomit at any time.

The chemical findings in blood and urine are shown in Table III and Chart 3.

TABLE III.

Chemical Findings in Blood and Urine of Case 3.

	Day after operation	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
BLOOD (Mgms. per 100 c.c.)	Non-protein Nitrogen	91	71		61	54	42	42		51			53			34	
	Urea Nitrogen		37		34	24		14		12			12			14	
	Sugar	133	167		94	90	147	222		182			154			110	
	Uric Acid		2.6		2.1	3.3	2.5			3.1			2.0			3.0	
	Creatinine		1.9		1.9	1.7	1.4	1.6		1.9			2.0			1.9	
	CO_2 Combining Power		65		59	54	53	54		53			60			52	
	Chlorides (Whole blood)		480		520	465		520		360			470			470	
URINE Gms. per day	Nitrogen			21	33	13	10	7.5	4.4	12		20	1.6	6.2	2.5	1.8	5.0
	Chlorides			0.6	0.9	0.7	1.8	0.6	1.1	2.4		4.4	3.5	8.0	5.6	1.7	4.5



DISCUSSION

These three patients show a marked increase in the non-protein nitrogenous bodies of the blood in general, proportional to the severity of the intoxication. The two patients on whom chemical studies of the urine were made, showed, with no food intake, an enormous excretion of nitrogen. One patient excreted 28 grams and the other 33 grams of nitrogen in twenty-four hours at the height of the intoxication. This great increase in urinary nitrogen could be due only to considerable breaking down of body protein. The destruction of tissue explains also the rise in non-protein nitrogen of the blood. It is of interest in this connection to note that the urine of Case 3, on the eleventh day following operation, showed large numbers of tyrosine and leucine crystals.

The renal function of the first patient was very carefully worked out before operation. The qualitative examination of the urine was negative. The concentration test, phenolsulphonephthalein test, and blood chemistry were normal. The other two patients showed no evidence of a renal lesion. Renal insufficiency can, we believe, be ruled out as a cause for the high nitrogen level in the blood.

Case 1 presented a marked alkalosis as evidenced by the very high CO_2 combining power of the blood plasma. She also showed clinical symptoms of alkalosis, the most marked being sighing respiration. The bicarbonate content of the blood was not determined in the other two cases until the acute stage of the intoxication had passed. It was then at the high level of normal. Another patient, with lower intestinal tract obstruction, studied recently, but whose case is not reported in this series, has shown a well marked alkalosis. The urine in Cases 2 and 3 was very alkaline.

The two cases in which the urine was studied showed a very striking suppression of chlorides. The chlorides re-appeared almost as if by crisis, ten days after the onset of symptoms. Coincident with the low level of chloride excretion, there was a very low chloride content of both plasma and whole blood. We are inclined to interpret the small chloride output and the low level of chloride in the blood as a part of and due to the action of the toxic substances. The findings bear a striking resemblance to the chloride metabolism in pneumonia which Whipple [7] suggests as possibly due to a proteose intoxication.

The alkalosis is evidently closely related to the chloride metabolism. It is probably simply an incident in the general intoxication. It seems quite possible that the chlorine is fixed or utilized somewhere in the course of the intoxication, probably in the process of the protein destruction. This leaves the sodium ion free to combine with the CO_2 and circulate as sodium bicarbonate with the production of an alkalosis. The vomiting in the first case may account for the loss of some chlorine, but this was not marked and in one of the other cases studied, in which no vomiting occurred, (Case 3), there was still an alkalosis with low blood chlorides and an absence of chlorides in the urine. All the patients were given some sodium bicarbonate before the nature of the process was manifest, but in no case was enough taken to produce the level of bicarbonate found.

The alkalosis and chloride metabolism present very interesting and important features of the intoxication due to intestinal obstruction. If our idea that the chlorine is actually utilized during the process of protein destruction be correct, it seems quite possible that the phenomenon may be a protective one. The provision of a sufficient amount of chloride to keep the blood chlorides at the normal level may be effective in combating the intoxication. Experiments on dogs from the standpoint of the rôle of the chlorides in such intoxications are already under way and will be reported in a subsequent paper.

It was proven by X-Ray in the first patient that there was no interruption of continuity below the gastroenterostomy stoma. Severe acute symptoms following gastroenterostomy have heretofore been ascribed to an actual obstruction in the jejunum distal to the stoma. There was no reason to suspect any obstruction in the other two cases. It is of interest to note that the first patient had evidence of an abnormal condition of the duodenum before operation. It seems quite possible that the periodic attacks, from which the patient suffered, may have been due to an intoxication from the duodenum. The fundamental cause of the disturbance is probably an obstruction to the outflow of duodenal contents, thus allowing an opportunity for the formation of toxic substances in a portion of the intestine in which absorption is rapid and complete. Here a condition is

produced similar to that in the experiment of Whipple cited above.

Murchison [8] had reported a case of diverticulum of the duodenum. The patient succumbed following excision. This patient was also seen by one of us (R. L. H.). He presented the clinical picture of an extreme intoxication. The blood urea nitrogen rose to 86.9 milligrams per 100 c.c. of blood. We believe that this patient died from an intoxication similar to that from which the patients here reported suffered.

Summary

Three cases are reported showing a high level of non-protein nitrogen of the blood, low blood chlorides and suppression of chloride excretion following gastroenterostomy.

One patient had a marked alkalosis; two had a CO_2 combining power at the upper limit of normal when first determined.

Two patients also had a very high nitrogen excretion in the urine.

All three patients presented clinical symptoms of a severe intoxication.

The clinical picture and laboratory findings are believed to be due to an intoxication arising in the upper intestinal tract.

The fundamental cause in these cases is probably a duodenal obstruction at the site of the gastroenterostomy which interferes with drainage of the duodenal loop.

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A SELECTIVE CULTURE MEDIUM FOR THE DIPHTHERIA BACILLUS

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The formula of Loeffler gives excellent results as a culture medium for the diphtheria bacillus but, as it is not ideal, many attempts have been made to improve upon it, so far without much success. Loeffler's medium is still regarded as the best for routine diagnostic procedures, and is used extensively for Public Health work in this country and abroad. At the suggestion of Dr. C. G. Guthrie the author undertook the elaboration of a more selective medium for the diphtheria bacillus.

It was planned to search for a substance or substances which would accelerate the growth of the diphtheria bacillus, and at the same time inhibit the development of the ordinary organisms commonly found in association with it in cultures from the throat. If a substance possessing both of these properties should not be found, a substance exhibiting either of them would be welcomed.

To be really "selective," an inhibitory substance should not be merely relative in its action; that is, while inhibiting the growth of other organisms, it should not exert any depressant effect on the organism it is desired to isolate; but such substances are rare. The inhibitory power of some of the substances—such as bile and sodium oleate—which have been used in "selective" media for certain other bacteria is neutralized by the presence of serum. Fearing that it might prove necessary to utilize a serum-free base, comparative tests were made of the growth of the diphtheria bacillus upon Dorset's egg medium, Vedder's starch agar, glycerinated agar, and hæmoglobin agar. On all of these, with the exception of the egg medium the growth was found to be inferior to that on Loeffler's serum. These and other experiments led to the conclusion—in which practically all workers in this subject have agreed—that serum is one of the best growth-promoting substances for the diphtheria bacillus.

The influence of the reaction of the medium was next studied. It was found that the diphtheria bacillus is not very fastidious in this respect; its range of growth is wide. When agar was used as a base ("hormone agar" of Huntoon), and to this various amounts of acid and alkali were added, it was found that growth begins at pH. 6.1, and continues until pH. 8.7 is reached; the best growth, however, occurs between pH. 7.3 and pH. 7.9. Taking pH. 7.8 as representing neutrality to phenolphthalein, it is seen that the growth range of the diphtheria bacillus extends somewhat further on the acid than on the alkaline side. In studying the effects of acids and alkalies on the growth of the diphtheria bacillus in broth cultures, Cobbett [1] found that good growth took place with as much as 30 c.c. of normal alkali per litre, but cessation of growth occurred with 40 c.c. of normal alkali. It took only 7.5 c.c. of normal acid per litre, however, to inhibit its growth, cessation occurring with 12.5 c.c. The hydrogen-ion concentration of his broth was not determined, as this work appeared in 1897.

Having decided to avoid, for the time being, the use of serum as a growth-promoting substance on account of its possible antagonistic action to the selective substance to be used, an attempt was made to reinforce the medium by the use of certain vegetable and animal extracts,—loosely spoken of as "vitamines." After the author had experimented with such substances as onion juice, potato juice and tomato juice, an article by Leichtentritt [2] appeared, in which he discussed the effect of certain vegetable extracts on the growth of the staphylococcus and the diphtheria bacillus. He found that malt extract or lemon juice added to agar greatly enhanced the growth of a pure culture of the diphtheria bacillus. He concluded, after testing such a medium by culturing the throats of a series of patients suffering from diphtheria, that while it gave no better results than Loeffler's serum, it had the advantage of being easily sterilized and of being transparent. Based upon this suggestion, experiments with lemon juice were performed. First the juice was passed through a Berkefeld filter—without addition of alkali as Leichtentritt recommended—and added to veal infusion broth in graded concentrations. This fluid medium was inoculated with a mixture of diphtheria bacilli and other throat organisms. The tubes were incubated for 6 to 8 hours, and then streaked on blood-agar plates. The diphtheria bacilli grew out in much larger colonies than they usually do on blood-agar, which is but an indifferent medium for this organism. In addition, there was apparently some inhibition of the other bacteria contained in the mixture.

It then seemed preferable to work with pure cultures, and six organisms were used; *B. diphtheriæ*, *Streptococcus viridans*, *Streptococcus hæmolyticus*, *Pneumococcus*, a member of the *Micrococcus catarrhalis* group and *Staphylococcus albus*. All of these had been recently isolated from diphtheria throats, and represented the composite flora of several persons; they were used in broth suspensions of equal and only moderate density. A series of agar plates containing graded amounts of lemon juice was prepared, each of these was divided into six parts, and the six organisms streaked on the surface. After incubation for 24 hours a rather striking result was obtained. As the concentration of the lemon juice increased, one organism after another dropped out, until, on one plate containing a large amount of lemon juice, only the diphtheria bacillus grew. The experiment was repeated with lemon juice that had been autoclaved at 30 pounds pressure for half an hour, and the same striking differential effect was obtained. The antiscorbutic vitamine in lemon juice does not withstand so much heat as this.

The growth of the diphtheria bacillus on this lemon juice agar was not luxuriant, however, at least not so luxuriant as on Loeffler's serum, and it was felt that there might be an improvement in this respect, if serum could be incorporated in the medium, provided the serum would not interfere with the differential effect of the lemon juice. Accordingly sterile ascitic fluid was added in proportions ranging from ⅓ to ⅘ of the total volume, and it was found that the growth of the diphtheria bacillus was much more luxuriant, while the differential effect of the lemon juice was just as pronounced as before.

This medium seemed admirably adapted for diagnostic purposes except for the trouble involved in obtaining sterile human serum or ascitic fluid in sufficient quantity whenever it might be needed; hence experiments were made with animal serum. Three parts of pig serum and one part of veal infusion broth were mixed as for Loeffler's medium, and to this graded amounts of lemon juice were added. The fluid medium was tubed, coagulated and subjected to fractional sterilization in the usual way. With it the same differential effect was observed, one organism after another dropping out until *B. diphtheriæ alone* remained. Moreover, the diphtheria bacillus seemed to grow more luxuriantly than on the Loeffler's serum control, although no strictly quantitative estimations of growth were made at this time.

What is the substance in lemon juice that has the rather remarkable property of inhibiting five common throat organisms and, in the lower concentrations, of accelerating the growth of *B. diphtheriæ*? Citric acid is the most obvious, as it is present in large amount; it accounts for 80.95 per cent of the total acidity of lemon juice and for 4 to 5 per cent of the volume. There are likewise present other organic acids, such as tartaric and oxalic; certain saccharides and albuminous substances are also obtained on analysis. Experiments with solutions of citric acid gave results similar to those obtained with lemon juice.

A more careful study was made of the effect of citric acid on the development of the six organisms used in the earlier experiments. Loeffler's serum plates containing graded amounts of citric acid were prepared and each plate was divided into four quadrants. Four suspensions of diphtheria bacilli in salt solution were prepared, the first one slightly opalescent, the second and third less so, and the fourth containing so few bacteria that the fluid was clear and none could be seen in stained films. These four suspensions of *B. diphtheriæ* were streaked on the divided plates, as well as on plates of plain Loeffler's serum, and incubated for 12 to 16 hours. A distinct and appreciable increase in the growth of *B. diphtheriæ* was observed on the first 3 or 4 plates. This was evident in two ways: (1) the Loeffler's serum plates showed growth only from the two denser suspensions and none from the two more dilute suspensions, but the citric acid plates showed growth from the first three suspensions. (2) The relative luxuriance of growth was much greater on the citric acid plates. This experiment showed that a definite growth-accelerating action for the diphtheria bacillus was present. The experiment was repeated several times but always with the same general result.

Upon what does the differential inhibitory action of citric acid depend? It was suspected that this was probably a matter of hydrogen-ion concentration; in other words, that the diphtheria bacillus had a wider range of growth, and so could withstand more acid than could the other throat organisms. In order to test this theory, the following experiment was performed. A series of Loeffler's serum plates were prepared and the reaction graded by the use of N/1 HCl, so as to present variations from pH. 8.4 to pH. 5.5 The plates were streaked with pure cultures of the six organisms used in previous experiments, and then incubated. The same differential effect was observed that had been seen on the citric acid and lemon juice plates even to the order of disappearance of the various organisms: *Pneumococcus* usually dropped out first; then *Streptococcus viridans* and *Micrococcus catarrhalis*, then *Streptococcus hæmolyticus*, and finally *Staphylococcus albus*. It appears that *Staphylococcus aureus* is much more resistant to the effect of acid than *Staphylococcus albus;* at present it does not seem that the former can be successfully inhibited without also inhibiting the growth of the diphtheria bacillus; some strains of *Staphylococcus albus* also may be rather difficult to inhibit for the same reason.

Upon what does the growth-accelerating effect depend? Observing the growth-accelerating effect of lemon juice and certain vegetable extracts on the diphtheria bacillus and having excluded certain sugars as the possible cause, Leichtentritt concluded that the action was due to the presence of growth-accessory substances or "vitamines," but it is evident that in citric acid no vitamine is concerned. It is probably not dependent upon catalytic action, as the citric acid seems to act in a definitely quantitative manner; up to a certain point the amount of growth of the diphtheria bacillus increases with the increased amount of citric acid in the medium. It seems probable that this growth-accelerating effect is due to the fact that citric acid is a foodstuff which can be utilized as a source of carbon by the diphtheria bacillus. It has been shown that certain other organisms can readily split and utilize citric acid. To prove this point so far as the diphtheria bacillus is concerned, quantitative determinations were attempted, with a view to ascertaining whether the growth of the diphtheria bacillus in broth would cause a diminution in the amount of citric acid. In a small series of analyses it was found that *B. diphtheriæ* and *Streptococcus hæmolyticus* utilized citric acid in broth as they would a foodstuff, such as glucose. The number of analyses was small, however, and the method far from satisfactory when used with bacterial cultures.

Whether or not citric acid stands alone among organic acids in this growth-accelerating action on the diphtheria bacillus, has not been determined. It would be interesting to ascertain what other bacteria can utilize citric acid in the same way. The principle of acid inhibition is well known, but so far as I am aware, this is the first observation that an organic acid possessing this inhibitory action for other bacteria can act as a source of energy for the organism it is desired to isolate.

The salts of citric acid seem also to have a similar but less pronounced accelerating action on the growth of *B. diphtheriæ*. Sodium citrate has been used in connection with citric acid and the new medium tested in the following manner. Throat cultures were taken from 10 convalescent diphtheria patients who had had one or two negative cultures; 3 were positive on the citric acid medium and negative on Loeffler's serum; 2 were positive on both; and 5 were negative on both.

The method of preparation of the medium is quite simple. To 75 c.c. of clear pig, sheep or human serum, is added 1 c.c. of a 50 per cent sodium citrate solution and sufficient dextrose-veal-infusion-broth to bring the volume to 100 c.c. With a 3 per cent citric acid solution the reaction is then adjusted to pH. 6.4 by the colorimetric method, as described in recent editions of books on bacteriological technic. It has been found that certain indicators, as for instance brom-cresol-purple, give a qualitative error in the presence of a large amount of protein; that is, colors may be seen which cannot be properly matched with the standards. Di-brom-thymol-blue is an indicator which covers the range required, gives fairly good colors and is not subject to this qualitative error. The 3 per cent citric acid solution is diluted 1:10 for the preliminary colorimetric titration, but is used undiluted when added for adjustment of the reaction. If the medium is made more acid than pH. 6.4, it does not coagulate firmly and the surface may break when it is streaked with a platinum loop or throat swab. It is desirable, however, to have the reaction as acid as possible without interfering with the coagulability of the medium, so that the maximal inhibitory effect upon other throat organisms may be obtained; the optimum seems to be pH. 6.4. After the reaction has been adjusted with citric acid, the medium is delivered into culture tubes, coagulated in a Koch inspissator and sterilized by the fractional method on three successive days. In many laboratories Loeffler's serum is made with beef extract broth instead of meat infusion broth; this is much cheaper and probably does not appreciably impair the quality of the medium; it can be used for this medium also. The preparation of the medium may be simplified further by inspissating for one period of four hours instead of by the fractional method.

The morphology of the diphtheria bacillus grown on the citric acid medium does not differ materially from that on Loeffler's serum. The macroscopic appearance of the growth always shows well marked yellowish coloration, and greater luxuriance than on Loeffler's serum; otherwise the appearance of the growth is very similar on the two media. There is no indication that this medium serves to inhibit the growth of closely allied organisms such as *B. xerosis* and *B. hofmanni* or to

Fig. 1.

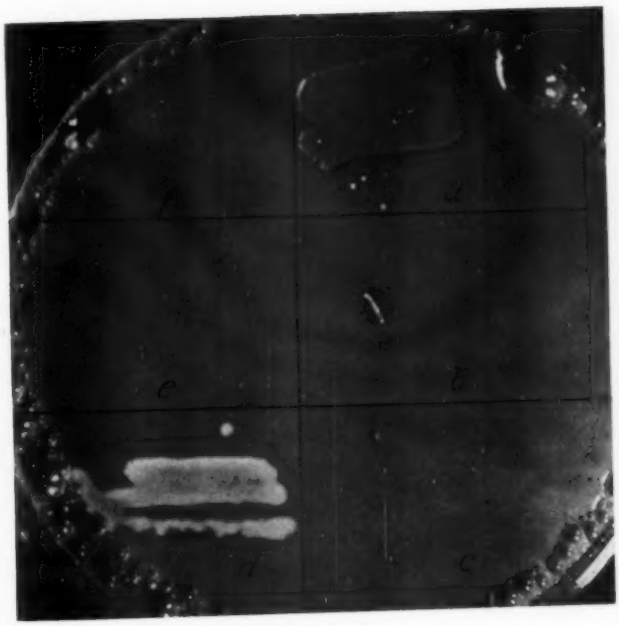


Fig. 2.

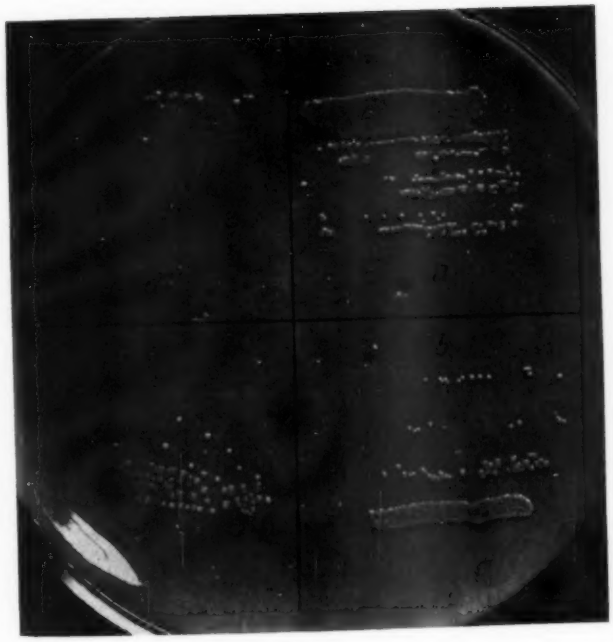
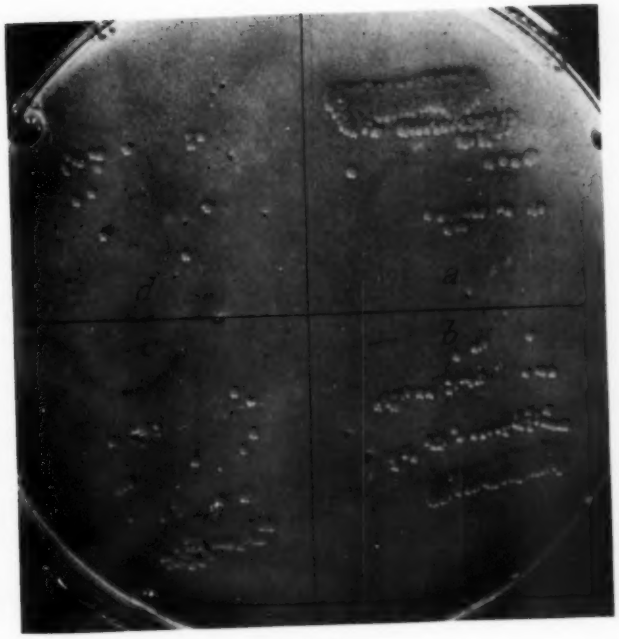


Fig. 3.



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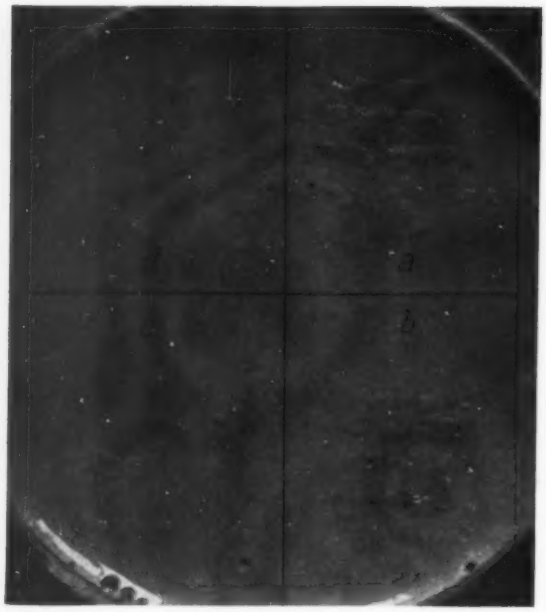

Fig. 5.

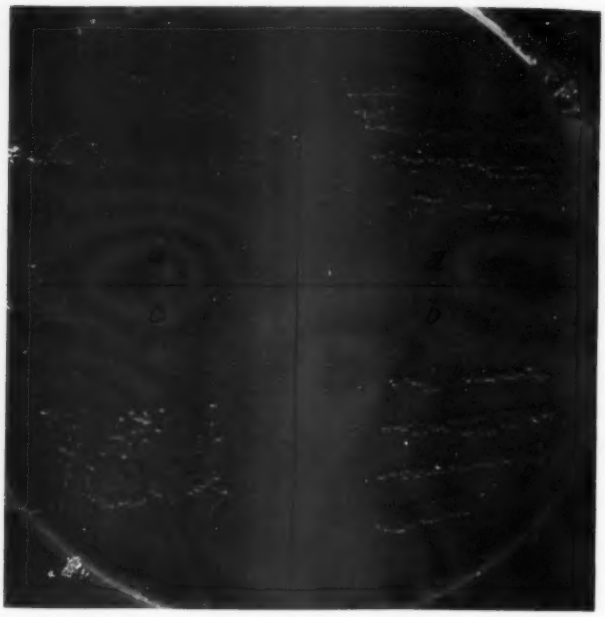

Fig. 6.

Fig. 7.

distinguish them from *B. diphtheriæ*. Observations made so far seem to indicate that growth on this citric acid medium does not diminish the virulence of diphtheria bacilli.

It is felt that this medium has definite advantages over Loeffler's serum for diagnostic purposes and that it is particularly adapted for the isolation of the diphtheria bacillus when pure cultures are required for virulence tests or other purposes.

SUMMARY

The reaction of the serum medium herein described is adjusted to pH. 6.4 by means of citric acid. The citric acid plays a dual rôle in that (1) it has a differential effect, inhibiting many of the common throat organisms which cannot grow at this pH; and (2) it has a growth-accelerating effect on the diphtheria bacillus to which it apparently serves as a source of carbon. Tests made with this medium show it to be definitely superior to Loeffler's serum for the cultivation and isolation of the diphtheria bacillus.

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LEGENDS FOR FIGURES

Fig. 1.—Loeffler's serum plate streaked with:
- (a) *B. diphtheriæ.*
- (b) *Streptococcus viridans.*
- (c) *Streptococcus hæmolyticus.*
- (d) *Staphylococcus aureus.*
- (e) Member of *Micrococcus catarrhalis* group.
- (f) *Pneumococcus.*

Photograph taken at an angle to show *Pneumococcus* and *M. catarrhalis* which are seen only by transmitted light.

Fig. 2.—Citric-serum plate streaked with the same six organisms, in the same order and in the same manner as in Fig. 1. Shows inhibition of all but *B. diphtheriæ* and *Staphylococcus aureus.*

Fig. 3.—*B. diphtheriæ* on Loeffler's medium, and

Fig. 4.—*B. diphtheriæ* on citric-serum medium, pH. 6.4. Shows quantitative growth from bacterial suspensions of decreasing density. The increase in the number and size of colonies in (c) and (d) on the citric plate can be seen.

Figs. 5, 6, 7.—Loeffler's serum plate adjusted to pH. 6.4. by:
- N/1 HCl (Fig. 5).
- Berkefeld filtered lemon juice (Fig. 6).
- 3 per cent citric acid (Fig. 7).

Plates were streaked with a freshly isolated culture of *B. diphtheriæ* in the same manner as in previous experiments (Figs. 3 and 4); Fig. 6 shows an increase in growth over Fig. 5, and Fig. 7 shows increase over Fig. 6.

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